Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA05/000040

International filing date: 12 January 2005 (12.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/535,496
Filing date: 12 January 2004 (12.01.2004)

Date of receipt at the International Bureau: 23 February 2005 (23.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

PCT/CA 20 05/000040

07 FEBRUARY 2005 07.02.05

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APPLICATION NUMBER: *60/535,496*
FILING DATE: *January 12, 2004*

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PTO/SB/16 (08-03)
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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

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Additional inventors are being named on the ______ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Antisense Oligonucleotides Directed Against Ribonucleotide Reductase R2 And Uses Thereof In The Treatment Of Cancer

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS (check all that apply)

☑ Specification Number of Pages 57
☑ Drawing(s) Number of Sheets 16
☐ Application Date Sheet. See 37 CFR 1.76
☐ CD(s), Number ______
☐ Other (specify) ______

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[Page 1 of 2]

Respectfully submitted,
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Docket Number: 683-130PR

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ANTISENSE OLIGONUCLEOTIDES DIRECTED TO RIBONUCLEOTIDE REDUCTASE R2 AND USES THEREOF IN THE TREATMENT OF CANCER

FIELD OF THE INVENTION

The present invention pertains to the field of cancer therapeutics and in particular to combinations of an antisense oligonucleotide and one or more chemotherapeutic drugs for the treatment of cancer.

BACKGROUND

Regulation of ribonucleotide reductase, and particularly the R2 component, is altered in malignant cells exposed to some tumour promoters and to the growth factor TGF-β [Amara, et al., 1994; Chen et al., 1993; Amara et al., 1995b; Hurta and Wright, 1995; Hurta et al., 1991]. Higher levels of enzyme activity have been observed in cultured malignant cells when compared to nonmalignant cells [Weber, 1983; Takeda and Weber, 1981; Wright et al., 1989a], and increased levels of R2 protein and R2 mRNA have been found in pre-malignant and malignant tissues as compared to normal control tissue samples [Saeki et al., 1995; Jensen et al., 1994]. However, these correlative studies did not show a direct role for ribonucleotide reductase in cancer cell transformation and tumor progression, because like so many other enzyme activities found to be altered in cancer cells [e.g. Weber, 1983], the results could easily be explained by the increased cell proliferation and altered cell cycle regulation characteristics of transformed and malignant cell populations [Morgan and Kastan, 1997].

Antisense oligonucleotides directed to the R1 or R2 component of ribonucleotide reductase have been shown to be effective in reducing the growth of cancer cells [see, for example, U.S. Patent Nos. 5,998,383 and 6,121,000].

In view of the high incidence of various types of cancer throughout the world, there remains a need for improved therapies for the treatment of cancer.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention. Publications referred to throughout the specification are hereby incorporated by reference in their entireties in this application.

SUMMARY OF THE INVENTION

An object of the present invention is to provide antisense oligonucleotides directed to ribonucleotide reductase R2 and uses thereof in the treatment of cancer. In accordance with an aspect of the present invention, there is provided a use of an antisense oligonucleotide comprising SEQ ID NO:1 in combination with capecitabine for the treatment of renal carcinoma.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts effects of combination therapy on HT-29 colon tumour growth in nude mice.

Figure 2 depicts effects of combination therapy on HT-29 colon tumour growth in nude mice.

Figure 3 depicts effects of combination therapy on Caki-1 renal tumour growth in SCID mice.

Figure 4 depicts effects of combination therapy on prostatic tumour growth in SCID mice.

Figure 5 depicts effects of combination therapy on prostatic tumour growth in SCID mice.

Figure 6 depicts effects of combination therapy on A2058 melanoma growth in nude mice.

Figure 7 depicts effects of combination therapy on breast tumour growth in CD-1 nude mice.

Figure 8 depicts effects of combination therapy on ovary tumour growth in CD-1 nude mice.

Figure 9 depicts effects of GTI-2040 in the treatment of human pancreatic carcinoma in CD-1 nude mice.

Figure 10 depicts effects of GTI-2040 in the treatment of human cervix epitheloid carcinoma resistant to hydroxyurea (HU) in SCID mice.

Figure 11 depicts effects of GTI-2040 in the treatment of human breast adenocarcinoma resistant to cisplatin in SCID mice.

Figure 12 depicts effects of GTI-2040 in the treatment of human breast adenocarcinoma resistant to cisplatin in SCID mice.

Figure 13 depicts effects of GTI-2040 in the treatment of human breast adenocarcinoma resistant to taxol in SCID mice.

Figure 14 depicts effects of GTI-2040 in the treatment of human breast adenocarcinoma resistant to taxol in SCID mice.

Figure 15 depicts effects of GTI-2040 in the treatment of human promyelocytic leukemia resistant to taxol in SCID mice.

Figure 16 depicts effects of GTI-2040 in the treatment of LS513, human multi-drug resistant colon adenocarcinoma in SCID mice.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to combinations of an antisense oligonucleotide against the gene encoding a mammalian ribonucleotide reductase R2 protein and one or more chemotherapeutic agents. The combinations of the present invention are useful in the treatment of cancer. The combination of the antisense oligonucleotide with one or more chemotherapeutic agents has been found to be more effective in decreasing the growth and/or metastasis of cancer cells, including drug resistant cancer cells, than treatment with the antisense oligonucleotide or the chemotherapeutic agent(s) alone.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

The term "antisense oligonucleotide" as used herein means a nucleotide sequence that is complementary to the mRNA for the desired gene. In the context of the present invention, the desired gene is the gene encoding a mammalian ribonucleotide redustase R2 protein.

The term "selectively hybridise" as used herein refers to the ability of a nucleic acid to bind detectably and specifically to a second nucleic acid. Oligonucleotides selectively hybridise to target nucleic acid strands under hybridisation and wash conditions that minimise appreciable amounts of detectable binding to non-specific nucleic acids. High stringency conditions can be used to achieve selective hybridisation conditions as known in the art and discussed herein.

Typically, hybridisation and washing conditions are performed at high stringency according to conventional hybridisation procedures. Washing conditions are typically 1-3 x SSC, 0.1-1% SDS, 50-70°C with a change of wash solution after about 5-30 minutes.

The term "corresponds to" as used herein with reference to nucleic acid sequences means a polynucleotide sequence that is identical to all or a portion of a reference polynucleotide sequence. In contradistinction, the term "complementary to" is used herein to mean that the polynucleotide sequence is identical to all or a portion of the complement of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used herein to describe the sequence relationships between two or more polynucleotides: "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity." A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (*i.e.* a portion of the complete

polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity.

A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotide positions wherein a polynucleotide sequence may be compared to a reference sequence of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.* gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* (U.S.A.) 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 573 Science Dr., Madison, WI), or by inspection, and the best alignment (*i.e.* resulting in the highest percentage of identity over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide sequences are identical (*i.e.* on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.* A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.* the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 30 percent sequence identity, often at least 50 percent sequence identity, and more usually at least 60 percent sequence

identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, and frequently over a window of at least 25-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

ANTISENSE MOLECULES

Selection and Characteristics

It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of the present invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is the gene encoding a mammalian ribonucleotide reductase R2 protein. The sequences of various mammalian ribonucleotide reductase genes are known in the art, for example, the sequence for the human ribonucleotide reductase R2 gene is provided in Pavloff *et al.* [*J. DNA sequencing and Mapping,* 2;227-234 (1992)]. This and other mammalian R2 sequences are also available from the GenBank database maintained by the NCBI.

The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, *e.g.* detection or modulation of expression of the protein encoded by the gene, will result. Once the target site or sites have been identified, oligonucleotides are chosen that are sufficiently complementary (*i.e.* hybridise with sufficient strength and specificity) to the target to give the desired result.

Generally, antisense oligonucleotides are targeted to the 5' untranslated region (5'-UTR), the translation initiation or start codon region, the open reading frame (ORF), the translation termination or stop codon region or the 3' untranslated region (3'-UTR) of a gene. In accordance with the present invention, the antisense oligonucleotide is targeted to part of the open reading frame region of the gene.

The antisense oligonucleotides in accordance with the present invention are selected from a sequence complementary to the ribonucleotide reductase R2 gene such that the sequence exhibits the least likelihood of forming duplexes, hair-pins, or of containing homooligomer / sequence repeats. The oligonucleotide may further contain a GC clamp. One skilled in the art will appreciate that these properties can be determined qualitatively using various computer modelling programs, for example, the program OLIGO® Primer Analysis Software, Version 5.0 (distributed by National Biosciences, Inc., Plymouth, MN).

It is understood in the art that an antisense oligonucleotide need not have 100% identity with the complement of its target sequence. The antisense oligonucleotides in accordance with the present invention have a sequence that is at least about 75% identical to the complement of target sequence. In one embodiment of the present invention, the antisense oligonucleotides have a sequence that is at least about 90% identical to the complement of the target sequence. In a related embodiment, they have a sequence that is at least about 95% identical to the complement of target sequence, allowing for gaps or mismatches of several bases. Identity can be determined, for example, by using the BLASTN program of the University of Wisconsin Computer Group (GCG) software or provided on the NCBI website.

In order to be effective, antisense oligonucleotides are typically between 7 and 100 nucleotides in length. In one embodiment of the present invention, the antisense oligonucleotides are between about 7 to about 50 nucleotides in length. In other embodiments, the antisense oligonucleotides are between about 7 to about 35 nucleotides in length, between about 15 to about 25 nucleotides in length, and about 20 nucleotides in length.

The antisense oligonucleotides of the present invention comprise at least 7 contiguous nucleotides, or nucleotide analogues that correspond to a part of the coding region of a mammalian ribonucleotide reductase R2 gene.

Suitable antisense oligonucleotides for use in the combinations of the present invention include those disclosed in U.S. Patent Nos. 5,998,383 and 6,121,000 (herein incorporated by reference) which are targeted to the ribonucleotide reductase R2 gene. In one embodiment of the present invention, the antisense oligonucleotide comprises at least 7 consecutive nucleotides, or nucleotide analogues selected from the antisense oligonucleotide sequence AS-II-626-20:

5'-GGCTAAATCGCTCCACCAAG-3' [SEQ ID NO:1]

The term "antisense oligonucleotides" as used herein includes other oligomeric antisense compounds, including oligonucleotide mimetics, modified oligonucleotides, and chimeric antisense compounds. Chimeric antisense compounds are antisense compounds that contain two or more chemically distinct regions, each made up of at least one monomer unit.

Thus, in the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), or RNA or DNA mimetics. This term, therefore, includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions, which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

As is known in the art, a nucleoside is a base-sugar combination and a nucleotide is a nucleoside that further includes a phosphate group covalently linked to the sugar portion of the nucleoside. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound, with the normal linkage or backbone of RNA and DNA being a 3' to 5' phosphodiester linkage. Specific examples of antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include both those that retain a phosphorus atom in the backbone and those that lack a phosphorus atom in the backbone. For the purposes of the present invention, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Exemplary modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates,

thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. In one embodiment of the present invention, the antisense oligonucleotide comprises at least one phosphorothioate linkage.

Exemplary modified oligonucleotide backbones that do not include a phosphorus atom are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. Such backbones include morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulphone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulphamate backbones; methyleneimino and methylenehydrazino backbones; sulphonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

The present invention also contemplates oligonucleotide mimetics in which both the sugar and the internucleoside linkage of the nucleotide units are replaced with novel groups. The base units are maintained for hybridisation with an appropriate nucleic acid target compound. An example of such an oligonucleotide mimetic, which has been shown to have excellent hybridisation properties, is a peptide nucleic acid (PNA) [Nielsen *et al.*, *Science*, 254:1497-1500 (1991)]. In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza-nitrogen atoms of the amide portion of the backbone.

Modified oligonucleotides may also contain one or more substituted sugar moieties. For example, oligonucleotides may comprise sugars with one of the following substituents at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Examples of such groups are: $O[(CH_2)_n O]_m CH_3$, $O(CH_2)_n OCH_3$, $O(CH_2)_n NH_2$, $O(CH_2)_n CH_3$, $O(CH_2)_n ONH_2$, and $O(CH_2)_n ON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10. Alternatively, the oligonucleotides may comprise one of the following substituents at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-

alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, $SO_2 CH_3$, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Specific examples include 2'-methoxyethoxy (2'-O--CH_2 CH_2 OCH_3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) [Martin *et al.*, *Helv. Chim. Acta,* 78:486-504(1995)], 2'-dimethylaminooxyethoxy ($O(CH_2)_2$ $ON(CH_3)_2$ group, also known as 2'-DMAOE), 2'-methoxy (2'-O--CH_3), 2'-aminopropoxy (2'-OCH_2 CH_2 CH_2 NH_2) and 2'-fluoro (2'-F). In one embodiment of the present invention, the antisense oligonucleotide comprises at least one nucleotide comprising a substituted sugar moiety. In another embodiment, the antisense oligonucleotide comprises at least one 2'-O-(2-methoxyethyl) or 2'-MOE modified nucleotide.

Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Oligonucleotides may also include modifications or substitutions to the nucleobase. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808; The Concise Encyclopedia Of Polymer Science And Engineering, (1990) pp 858-859, Kroschwitz, J. I., ed. John Wiley & Sons; Englisch *et al.*,

Angewandte Chemie, Int. Ed., 30:613 (1991); and Sanghvi, Y. S., (1993) *Antisense Research and Applications*, pp 289-302, Crooke, S. T. and Lebleu, B., ed., CRC Press. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C [Sanghvi, Y. S., (1993) *Antisense Research and Applications*, pp 276-278, Crooke, S. T. and Lebleu, B., ed., CRC Press, Boca Raton].

Another oligonucleotide modification included in the present invention is the chemically linkage to the oligonucleotide of one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, lipid moieties such as a cholesterol moiety [Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:6553-6556 (1989)], cholic acid [Manoharan *et al.*, *Bioorg. Med. Chem. Let.*, 4:1053-1060 (1994)], a thioether, *e.g.* hexyl-S-tritylthiol [Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 660:306-309 (1992); Manoharan *et al.*, *Bioorg. Med. Chem. Lett.*, 3:2765-2770 (1993)], a thiocholesterol [Oberhauser *et al.*, *Nucl. Acids Res.*, 20:533-538 (1992)], an aliphatic chain, *e.g.* dodecandiol or undecyl residues [Saison-Behmoaras *et al.*, *EMBO J.*, 10:1111-1118 (1991); Kabanov *et al.*, *FEBS Lett.*, 259:327-330 (1990); Svinarchuk *et al.*, *Biochimie*, 75:49-54 (1993)], a phospholipid, *e.g.* di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate [Manoharan *et al.*, *Tetrahedron Lett.*, 36:3651-3654 (1995); Shea *et al.*, *Nucl. Acids Res.*, 18:3777-3783 (1990)], a polyamine or a polyethylene glycol chain [Manoharan *et al.*, *Nucleosides & Nucleotides*, 14:969-973 (1995)], or adamantane acetic acid [Manoharan *et al.*, *Tetrahedron Lett.*, 36:3651-3654 (1995)], a palmityl moiety [Mishra *et al.*, *Biochim. Biophys. Acta*, 1264:229-237 (1995)], or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety [Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 277:923-937 (1996)].

One skilled in the art will recognise that it is not necessary for all positions in a given oligonucleotide to be uniformly modified. The present invention, therefore, contemplates the incorporation of more than one of the aforementioned modifications into a single oligonucleotide or even at a single nucleoside within the oligonucleotide. The present invention further includes antisense compounds that are chimeric compounds. These oligonucleotides typically contain at

least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridising to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridisation techniques known in the art.

An example of a suitable chimeric oligonucleotide would be an antisense oligonucleotides with a mixed phosphorothioate and 2'-O-methyl backbone. In one embodiment of the present invention, the antisense oligonucleotides comprise one or more phosphorothioate backbone linkages. In a another embodiment, all backbone linkages in the antisense oligonucleotide are phosphorothioate linkages.

In the context of the present invention, an antisense oligonucleotide is "nuclease resistant" when it has either been modified such that it is not susceptible to degradation by DNA and RNA nucleases or alternatively has been placed in a delivery vehicle which in itself protects the oligonucleotide from DNA or RNA nucleases. Nuclease resistant oligonucleotides include, for example, methyl phosphonates, phosphorothioates, phosphorodithioates, phosphotriesters, and morpholino oligomers. Suitable delivery vehicles for conferring nuclease resistance include, for example, liposomes. In one embodiment of the present invention, the antisense oligonucleotides are nuclease resistant.

The present invention further contemplates antisense oligonucleotides that contain groups for improving the pharmacokinetic properties of the oligonucleotide, or groups for improving the pharmacodynamic properties of the oligonucleotide.

Preparation of the Antisense Oligonucleotides

The antisense oligonucleotides of the present invention can be prepared by conventional techniques well-known to those skilled in the art. For example, the oligonucleotides can be prepared using solid-phase synthesis using commercially available equipment, such as the equipment available from Applied Biosystems Canada Inc., Mississauga, Canada. As is well-known in the art, modified oligonucleotides, such as phosphorothioates and alkylated derivatives, can also be readily prepared by similar methods.

Alternatively, the antisense oligonucleotides of the present invention can be prepared by enzymatic digestion of the naturally occurring ribonucelotide reductase R2 gene by methods known in the art.

Antisense oligonucleotides can also be prepared through the use of recombinant methods in which expression vectors comprising nucleic acid sequences that encode the antisense oligonucleotides are expressed in a suitable host cell. Such expression vectors can be readily constructed using procedures known in the art. Examples of suitable vectors include, but are not limited to, plasmids, phagemids, cosmids, bacteriophages, baculoviruses and retroviruses, and DNA viruses. One skilled in the art will understand that selection of the appropriate host cell for expression of the antisense oligonucleotide will be dependent upon the vector chosen. Examples of host cells include, but are not limited to, bacterial, yeast, insect, plant and mammalian cells.

One skilled in the art will also understand that the expression vector may further include regulatory elements, such as transcriptional elements, required for efficient transcription of the antisense oligonucleotide sequences. Examples of regulatory elements that can be incorporated into the vector include, but are not limited to, promoters, enhancers, terminators, and polyadenylation signals. One skilled in the art will appreciate that selection of suitable regulatory elements is dependent on the host cell chosen for expression of the antisense oligonucleotide and that such regulatory elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes.

In accordance with the present invention, the expression vectors can be introduced into a suitable host cell or tissue by one of a variety of methods known in the art. Such methods can be found generally described in Sambrook *et al.*, 1992; Ausubel *et al.*, 1989; Chang *et al.*, 1995; Vega *et*

al., 1995; and Vectors: A Survey of Molecular Cloning Vectors and Their Uses (1988) and include, for example, stable or transient transfection, lipofection, electroporation, and infection with recombinant viral vectors.

CHEMOTHERAPEUTIC AGENTS

The combinations provided by the present invention comprise an antisense oligonucleotide and one or more chemotherapeutic agents. A wide range of cancer chemotherapeutic agents is known in the art and includes those chemotherapeutic agents which are specific for the treatment of a particular type of cancer as well as those which may be applicable to a range of cancers, such as doxorubicin, capecitabine, mitoxantrone, irinotecan (CPT-11). The present invention contemplates the use of both types of chemotherapeutic agent in conjunction with the antisense oligonucleotides. Combination therapies using standard cancer chemotherapeutics are also well known in the art and may be used in conjunction with the antisense oligonucleotides.

Examples of chemotherapeutic agents suitable for the treatment of breast cancer include, but are not limited to, capecitabine, cyclophosphamide, ifosfamide, cisplatin, carboplatin, 5-fluorouracil (5-FU), taxol, taxanes such as paclitaxel and docetaxel and various anthracyclines, such as doxorubicin and epi-doxorubicin (also known as epirubicin). Combination therapies using standard cancer chemotherapeutics may also be used in conjunction with the antisense oligonucleotides and are also well known in the art, for example, the combination of epirubicin with paclitaxel or docetaxel, or the combination of doxorubicin or epirubicin with cyclophosphamide, which are used for breast cancer treatments. Polychemotherapeutic regimens are also useful and may consist, for example, of doxorubicin/cyclophosphamide/5-fluorouracil or cyclophosphamide/epirubicin/5-fluorouracil. Many of the above chemotherapeutics and combinations thereof are useful in the treatment of a variety of solid tumours.

Cyclophosphamide, mitoxantrone and estramustine are known to be suitable for the treatment of prostate cancer. Cyclophosphamide, vincristine, doxorubicin and etoposide are used in the treatment of small cell lung cancer, as are combinations of etoposide with either cisplatin or carboplatin. In the treatment of stomach or oesophageal cancer, combinations of doxorubicin or epirubicin with cisplatin and 5-fluorouracil are useful. For colorectal cancer, CPT-11 alone or in combination with 5-fluorouracil-based drugs, or oxaliplatin alone or in combinati n with 5-

fluorouracil-based drugs can be used. Oxaliplatin may also be usedin combination with capecitabine.

Other examples include the combination of cyclophosphamide, doxorubicin, vincristine and prednisone in the treatment of non-Hodgkin's lymphoma; the combination of doxorubicin, bleomycin, vinblastine and DTIC in the treatment of Hodgkin's disease and the combination of cisplatin or carboplatin with any one or a combination of gemcitabine, paclitaxel, docetaxel, vinorelbine or etoposide in the treatment of non-small cell lung cancer.

Other suitable chemotherapeutic agents include, but are not limited to, mitomycin C, vinblastine, novantrone, DTIC (dacarbzine), hydroxyurea and cytokines. Cytokines include but are not limited to interferons, interleukins such as IL-2-I, IL-2-II, and IL-12. Suitable combinations that include a cytokine may also include other of the chemotherapeutic agents discussed herein.

Examples of suitable combinations of the antisense oligonucleotide and one or more chemotherapeutic agent include, but are not limited to, a combination of the antisense oligonucleotide and capecitabine for the treatment of solid tumours, breast cancer, renal cancer or colorectal cancer; a combination of the antisense oligonucleotide, capecitabine and oxaliplatin for the treatment of colorectal cancer and pancreatic cancer; a combination of the antisense oligonucleotide and docetaxel for the treatment of solid tumours, including non-small cell lung carcinoma (NSCLC), prostate cancer and cancer of the genitourinary tract; a combination of the antisense oligonucleotide and gemcitabine for the treatment of solid tumours, NSCLC and renal cell carcinoma; a combination of the antisense oligonucleotide, gemcitabine and capecitabine for the treatment of colon cancer; a combination of the antisense oligonucleotide, gemcitabine and oxaliplatin for the treatment of breast cancer; a combination of the antisense oligonucleotide and idarubicin for the treatment of acute myeloid leukaemia (AML); a combination of the antisense oligonucleotide and Ara-C for the treatment of AML and chronic myeloid leukaemia (CML); a combination of the antisense oligonucleotide, mitoxantrone, etoposide and Ara-C for the treatment of AML; a combination of the antisense oligonucleotide, fludarabin, filgrastim and Ara-C for the treatment of CML; a combination of the antisense oligonucleotide, carboplatin and paclitaxel for the treatment of metastatic cancer; a combination of the antisense oligonucleotide and cisplatin for the treatment of head and neck cancer, oesophageal cancer and lung cancer; a combination of the antisense oligonucleotide, cisplatin and irinotecan for the treatment of small-

cell lung carcinoma (SCLC); a combination of the antisense oligonucleotide and irinotecan for the treatment of pancreatic adenocarcinoma, a combination of the antisense oligonucleotide and 5-FU for the treatment of cancer of the pancreas, gall bladder and biliary ducts, and a combination of the antisense oligonucleotide and one or more cytokines for the treatment of renal cancer, or breast cancer, AML, lung cancer (NSLC or SCLC), prostate or colon cancer, or a variety of solid tumors.

In one embodiment the combination of the antisense oligonucleotide and a cytokine (for example an interferon or an interleukin) may be used in the treatment of renal carcinoma, for example early-stage renal carcinoma.

USE OF THE COMBINATIONS OF THE PRESENT INVENTION

The combinations of the present invention comprising an antisense oligonucleotide against ribonucleotide reductase R2 and one or more chemotherapeutic agents can be used in the treatment of a variety of cancers. In accordance with the present invention, the combination is more effective in reducing the growth and/or metastasis of cancer cells than either the antisense oligonucleotide or the chemotherapeutic agent(s) alone. The combinations can also be used to effectively treat drug resistant tumours.

Examples of cancers which may be may be treated, stabilised, or prevented in accordance with the present invention include, but are not limited to leukaemia, carcinomas, adenocarcinomas, melanomas and sarcomas. Carcinomas, adenocarcinomas and sarcomas are also frequently referred to as "solid tumors," examples of commonly occurring solid tumors include, but are not limited to, cancer of the brain, breast, cervix, colon, head and neck, kidney, lung, ovary, pancreas, prostate, stomach and uterus, non-small cell lung cancer and colorectal cancer.

The term "leukaemia" refers broadly to progressive, malignant diseases of the blood-forming organs. Leukaemia is typically characterised by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow but can also refer to malignant diseases of other blood cells such as erythroleukaemia, which affects immature red blood cells. Leukaemia is generally clinically classified on the basis of (1) the duration and character of the disease – acute or chronic; (2) the type of cell involved – myeloid (myelogenous), lymphoid

(lymphogenous) or m nocytic, and (3) the increase or non-increase in the number of abnormal cells in the blood – leukaemic or aleukaemic (subleukaemic). Leukaemia includes, for example, acute nonlymphocytic leukaemia, chronic lymphocytic leukaemia, acute granulocytic leukaemia, chronic granulocytic leukaemia, acute promyelocytic leukaemia, adult T-cell leukaemia, aleukaemic leukaemia, aleukocythemic leukaemia, basophylic leukaemia, blast cell leukaemia, bovine leukaemia, chronic myelocytic leukaemia, leukaemia cutis, embryonal leukaemia, eosinophilic leukaemia, Gross' leukaemia, hairy-cell leukaemia, hemoblastic leukaemia, hemocytoblastic leukaemia, histiocytic leukaemia, stem cell leukaemia, acute monocytic leukaemia, leukopenic leukaemia, lymphatic leukaemia, lymphoblastic leukaemia, lymphocytic leukaemia, lymphogenous leukaemia, lymphoid leukaemia, lymphosarcoma cell leukaemia, mast cell leukaemia, megakaryocytic leukaemia, micromyeloblastic leukaemia, monocytic leukaemia, myeloblastic leukaemia, myelocytic leukaemia, myeloid granulocytic leukaemia, myelomonocytic leukaemia, Naegeli leukaemia, plasma cell leukaemia, plasmacytic leukaemia, promyelocytic leukaemia, Rieder cell leukaemia, Schilling's leukaemia, stem cell leukaemia, subleukaemic leukaemia, and undifferentiated cell leukaemia.

The term "sarcoma" generally refers to a tumor which originates in connective tissue, such as muscle, bone, cartilage or fat, and is made up of a substance like embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas include soft tissue sarcomas, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented haemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas include, for example, acral-lentiginous melanoma, amelanotic

melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colorectal carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, haematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, non-small cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma

telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

The term "carcinoma" also encompasses adenocarcinomas. Adenocarcinomas are carcinomas that originate in cells that make organs which have glandular (secretory) properties or that originate in cells that line hollow viscera, such as the gastrointestinal tract or bronchial epithelia. Examples include, but are not limited to, adenocarcinomas of the breast, lung, pancreas and prostate.

Additional cancers encompassed by the present invention include, for example, Hodgkin's Disease, Non-Hodgkin's lymphoma, multiple myeloma, neuroblastoma, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, gliomas, testicular cancer, thyroid cancer, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, mesothelioma and medulloblastoma.

In one embodiment of the present invention, the cancer is selected from the group of: solid tumours, renal cell carcinoma, breast cancer, NSCLC, acute myeloid carcinoma, colorectal cancer, prostate cancer, melanoma, ovarian cancer, pancreatic carcinoma, cervix epitheloid carcinoma, breast adenocarcinoma, human promyelocytic leukemia, or multidrug resistant versions thereof.

"Aggressive cancer," as used herein, refers to a rapidly growing cancer. One skilled in the art will appreciate that for some cancers, such as breast cancer or prostate cancer the term "aggressive cancer" will refer to an advanced cancer that has relapsed within approximately the earlier two-thirds of the spectrum of relapse times for a given cancer, whereas for other types of cancer, such as small cell lung carcinoma (SCLC) nearly all cases present rapidly growing cancers which are considered to be aggressive. The term can thus cover a subsection of a certain cancer type or it may encompass all of other cancer types. A "refractory" cancer or tumour refers to a cancer or tumour that has not responded to treatment. "Advanced cancer," refers to overt disease in a patient, wherein such overt disease is not amenable to cure by local modalities of treatment, such as surgery or radiotherapy. Advanced disease may refer to a locally advanced cancer or it may

refer to metastatic cancer. The term "metastatic cancer" refers to cancer that has spread from one part of the body to another. The later stages of cancer, when long-term survival is not anticipated, may be termed "end-stage cancer". Metastatic cancer includes advanced end-stage cancer.

It is contemplated that the present invention may be used at various stages in tumour development and progression, including in the treatment of advanced and/or aggressive neoplasias (*i.e.* overt disease in a subject that is not amenable to cure by local modalities of treatment, such as surgery or radiotherapy), metastatic disease, locally advanced disease and/or refractory tumours (*i.e.* a cancer or tumour that has not responded to treatment). Treatment of refractory cancer may be termed a "second-line treatment", and is a contemplated use of the present invention, in addition to first-line treatment.

"Primary therapy" refers to treatment upon the initial diagnosis of cancer in a subject. Exemplary primary therapies may involve surgery, a wide range of chemotherapies and radiotherapy. "Adjuvant therapy" refers to a therapy that follows a primary therapy and that is administered to subjects at risk of relapsing. Adjuvant systemic therapy is typically begun soon after primary therapy to delay recurrence, prolong survival or cure a subject. In the context of treatment overall, first-line treatment may or may not be chemotherapeutic. In the context of adjuvant chemotherapy, chemotherapeutic treatment may be considered first-line chemotherapeutic treatment.

It is contemplated that the compounds of the invention can be used alone or in combination with one or more other chemotherapeutic agents as part of an primary or adjuvant therapy. Combinations of the compounds of the invention and standard chemotherapeutics may act to improve the efficacy of the chemotherapeutic and, therefore, can be used to improve standard cancer therapies. This application is also important in the treatment of drug-resistant cancers that are not responsive to standard treatment. Drug-resistant cancers can arise, for example, from heterogeneity of tumour cell populations, alterations in response to chemotherapy and increased malignant potential. Such changes are often more pronounced at advanced stages of disease and have, in part, as an underlying cause, changes in genome/message stability.

PHARMACEUTICAL COMPOSITIONS

The antisense oligonucleotide may be administered as a pharmaceutical composition with an appropriate pharmaceutically physiologically acceptable carrier, diluent, excipient or vehicle. The pharmaceutical compositions may also be formulated to contain the antisense oligonucleotide and one or more other chemotherapeutic agents for concurrent administration to a patient.

The pharmaceutical compositions of the present invention may be administered orally, topically, parenterally, by inhalation or spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques.

The pharmaceutical compositions may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to methods known to the art for the manufacture of pharmaceutical compositions and may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with suitable non-toxic pharmaceutically acceptable excipients including, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, such as corn starch, or alginic acid; binding agents, such as starch, gelatine or acacia, and lubricating agents, such as magnesium stearate, stearic acid or talc. The tablets can be uncoated, or they may be coated by known techniques in order to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

Pharmaceutical compositions for oral use may also be presented as hard gelatine capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active compound in admixture with suitable excipients including, for example, suspending agents, such as sodium carboxymethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example, polyoxyethyene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example, hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol for example, polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example, polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or *n*-propyl *p*-hydroxy-benzoate, one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavouring agents may be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active compound in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin, or it may be a mixtures of these oils. Suitable emulsifying agents may be naturally-occurring gums, for example, gum acacia or gum tragacanth; naturally-occurring phosphatides, for example, soy bean, lecithin; or esters or partial esters derived from fatty acids and hexitol, anhydrides, for example, sorbitan monoleate, and condensation products of the said

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partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monoleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, and/or flavouring and colouring agents.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known art using suitable dispersing or wetting agents and suspending agents such as those mentioned above. The sterile injectable preparation may also be sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Acceptable vehicles and solvents that may be employed include, but are not limited to, water, Ringer's solution, lactated Ringer's solution and isotonic sodium chloride solution. Other examples are, sterile, fixed oils which are conventionally employed as a solvent or suspending medium, and a variety of bland fixed oils including, for example, synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "*Remington: The Science and Practice of Pharmacy,*" Gennaro, A., Lippincott, Williams & Wilkins, Philidelphia, PA (2000) (formerly "*Remingtons Pharmaceutical Sciences*").

EFFICACY OF THE COMPOSITIONS

1. In vitro *Testing*

Initial determinations of the efficacy of the compositions of the present invention may be made using *in vitro* techniques if required.

For example, the combinations can be tested *in vitro* by determining their ability to inhibit anchorage-independent growth of tumour cells. Anchorage-independent growth is known in the art to be a good indicator of tumourigenicity. In general, anchorage-independent growth is assessed by plating cells from an appropriate cancer cell-line onto soft agar and determining the

number of colonies formed after an appropriate incubation period. Growth of cells treated with the combinations can then be compared with that of cells treated with an appropriate control (as described above) and with that of untreated cells.

In one embodiment of the present invention, *in vitro* testing of the combinations is conducted in a human cancer cell-line. Examples of suitable cancer cell-lines for *in vitro* testing of the combinations of the present invention include, but are not limited to, non-small cell lung carcinoma cell-lines A549 and H1299, breast cancer cell-line MCF-7, colon cancer cell-lines CaCo, HCT116 and HT29, cervical cancer cell-line HeLa. Other examples of suitable cell-lines are known in the art.

If necessary, the toxicity of the combinations can also be initially assessed *in vitro* using standard techniques. For example, human primary fibroblasts can be treated *in vitro* with the oligonucleotide in the presence of a commercial lipid carrier such as lipofectamine. Cells are then tested at different time points following treatment for their viability using a standard viability assay, such as the trypan-blue exclusion assay. Cells are also assayed for their ability to synthesize DNA, for example, using a thymidine incorporation assay, and for changes in cell cycle dynamics, for example, using a standard cell sorting assay in conjunction with a fluorocytometer cell sorter (FACS).

2. In vivo *Testing*

The ability of the combinations to inhibit tumour growth or proliferation *in vivo* can be determined in an appropriate animal model using standard techniques known in the art (see, for example, Enna, *et al., Current Protocols in Pharmacology*, J. Wiley & Sons, Inc., New York, NY).

In general, current animal models for screening anti-tumour compounds are xenograft models, in which a human tumour has been implanted into an animal. Examples of xenograft models of human cancer include, but are not limited to, human solid tumour xenografts in mice, implanted by sub-cutaneous injection and used in tumour growth assays; human solid tumour isografts in mice, implanted by fat pad injection and used in tumour growth assays; experimental models of

lymphoma and leukaemia in mice, used in survival assays, and experimental models of lung metastasis in mice.

For example, the combinations can be tested *in vivo* on solid tumours using mice that are subcutaneously grafted bilaterally with a pre-determined amount of a tumour fragment on day 0. The animals bearing tumours are mixed before being subjected to the various treatments and controls. In the case of treatment of advanced tumours, tumours are allowed to develop to the desired size, animals having insufficiently developed tumours being eliminated. The selected animals are distributed at random into groups that will undergo the treatments or act as controls. Suitable groupings would be, for example, those receiving the combination of the invention, those receiving the antisense alone, those receiving the chemotherapeutic agent(s) alone and those receiving no treatment. Animals not bearing tumours may also be subjected to the same treatments as the tumour-bearing animals in order to be able to dissociate the toxic effect from the specific effect on the tumour. Chemotherapy generally begins from 3 to 22 days after grafting, depending on the type of tumour, and the animals are observed every day. The combinations of the present invention can be administered to the animals, for example, by bolus infusion. The different animal groups are weighed about 3 or 4 times a week until the maximum weight loss is attained, after which the groups are weighed at least once a week until the end of the trial.

The tumours are measured about 2 or 3 times a week until the tumour reaches a pre-determined size and / or weight, or until the animal dies if this occurs before the tumour reaches the pre-determined size / weight. The animals are then sacrificed and the tissue histology, size and / or proliferation of the tumour assessed.

For the study of the effect of the compositions on leukaemias, the animals are grafted with a particular number of cells, and the anti-tumour activity is determined by the increase in the survival time of the treated mice relative to the controls.

To study the effect of the combinations of the present invention on tumour metastasis, tumour cells are typically treated with the composition *ex vivo* and then injected into a suitable test animal. The spread of the tumour cells from the site of injection is then monitored over a suitable period of time by standard techniques.

In vivo toxic effects of the oligonucleotides can be evaluated by measuring their effect on animal body weight during treatment and by performing haematological profiles and liver enzyme analysis after the animal has been sacrificed.

Table 1: Examples of xenograft models of human cancer

Cancer Model	Cell Type
Tumour Growth Assay Human solid tumour xenografts in mice (sub-cutaneous injection)	Prostate (PC-3, DU145) Breast (MDA-MB-231, MVB-9) Colon (HT-29) Lung (NCI-H460, NCI-H209) Pancreatic (ASPC-1, SU86.86) Pancreatic: drug resistant (BxPC-3) Skin (A2058, C8161) Cervical (SIHA, HeLa-S3) Cervical: drug resistant (HeLa S3-HU-resistance) Liver (HepG2) Brain (U87-MG) Renal (Caki-1, A498) Ovary (SK-OV-3)
Tumour Growth Assay Human solid tumour isografts in mice (fat pad injection)	Breast: drug resistant (MDA-CDDP-S4, MDA-MB435-To.1)
Survival Assay Experimental model of lymphoma and leukaemia in mice	Human: Burkitts lymphoma (Non-Hodgkin's) (raji) Murine: erythroleukemia (CB7 Friend

Cancer Model	Cell Type
	retrovirus-induced)
Experimental model of lung metastasis in mice	Human: melanoma (C8161) Murine: fibrosarcoma (R3)

CLINICAL TRIALS IN CANCER PATIENTS

One skilled in the art will appreciate that, following the demonstrated effectiveness of the combinations of the present invention *in vitro* and in animal models, they should be tested in Clinical Trials in order to further evaluate their efficacy in the treatment of cancer and to obtain regulatory approval for therapeutic use. As is known in the art, clinical trials progress through phases of testing, which are identified as Phases I, II, III, and IV.

Initially the combinations will be evaluated in a Phase I trial. Typically Phase I trials are used to determine the best mode of administration (for example, by pill or by injection), the frequency of administration, and the toxicity for the compounds. Phase I studies frequently include laboratory tests, such as blood tests and biopsies, to evaluate the effects of a compound in the body of the patient. For a Phase I trial, a small group of cancer patients are treated with a specific dose of the antisense oligonucleotide and the one or more chemotherapeutic agent(s). During the trial, the dose is typically increased group by group in order to determine the maximum tolerated dose (MTD) and the dose-limiting toxicities (DLT) associated with the compound. This process determines an appropriate dose to use in a subsequent Phase II trial.

A Phase II trial can be conducted to evaluate further the effectiveness and safety of the combinations. In Phase II trials, the combination is administered to groups of patients with either one specific type of cancer or with related cancers, using the dosage found to be effective in Phase I trials.

Phase III trials focus on determining how a compound compares to the standard, or most widely accepted, treatment. In Phase III trials, patients are randomly assigned to one of two or more "arms". In a trial with two arms, for example, one arm will receive the standard treatment

(control group) and the other arm will receive treatment with the combination of the present invention (investigational group).

Phase IV trials are used to further evaluate the long-term safety and effectiveness of a compound. Phase IV trials are less common than Phase I, II and III trials and will take place after the combination has been approved for standard use.

Eligibility of Patients for Clinical Trials

Participant eligibility criteria can range from general (for example, age, sex, type of cancer) to specific (for example, type and number of prior treatments, tumor characteristics, blood cell counts, organ function). Eligibility criteria may also vary with trial phase. For example, in Phase I and II trials, the criteria often exclude patients who may be at risk from the investigational treatment because of abnormal organ function or other factors. In Phase II and III trials additional criteria are often included regarding disease type and stage, and number and type of prior treatments.

Phase I cancer trials usually comprise 15 to 30 participants for whom other treatment options have not been effective. Phase II trials typically comprise up to 100 participants who have already received chemotherapy, surgery, or radiation treatment, but for whom the treatment has not been effective. Participation in Phase II trials is often restricted based on the previous treatment received. Phase III trials usually comprise hundreds to thousands of participants. This large number of participants is necessary in order to determine whether there are true differences between the effectiveness of the combination of the present invention and the standard treatment. Phase III may comprise patients ranging from those newly diagnosed with cancer to those with extensive disease in order to cover the disease continuum.

One skilled in the art will appreciate that clinical trials should be designed to be as inclusive as possible without making the study population too diverse to determine whether the treatment might be as effective on a more narrowly defined population. The more diverse the population included in the trial, the more applicable the results could be to the general population, particularly in Phase III trials. Selection of appropriate participants in each phase of clinical trial is considered to be within the ordinary skills of a worker in the art.

Assessment of patients prior to treatment

Prior to commencement of the study, several measures known in the art can be used to first classify the patients. Patients can first be assessed, for example, using the Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) scale. ECOG PS is a widely accepted standard for the assessment of the progression of a patient's disease as measured by functional impairment in the patient, with ECOG PS 0 indicating no functional impairment, ECOG PS 1 and 2 indicating that the patients have progressively greater functional impairment but are still ambulatory and ECOG PS 3 and 4 indicating progressive disablement and lack of mobility.

Patients' overall quality of life can be assessed, for example, using the McGill Quality of Life Questionnaire (MQOL) (Cohen *et al* (1995) *Palliative Medicine* 9: 207-219). The MQOL measures physical symptoms; physical, psychological and existential well-being; support; and overall quality of life. To assess symptoms such as nausea, mood, appetite, insomnia, mobility and fatigue the Symptom Distress Scale (SDS) developed by McCorkle and Young ((1978) *Cancer Nursing* 1: 373-378) can be used.

Patients can also be classified according to the type and/or stage of their disease and/or by tumor size.

Administration of the combinations of the present invention in Clinical Trials

The antisense oligonucleotide and the one or more chemotherapeutic agent(s) are typically administered to the trial participants parenterally. In one embodiment, the combination is administered by intravenous infusion. Methods of administering drugs by intravenous infusion are known in the art. Usually intravenous infusion takes place over a certain time period, for example, over the course of 60 minutes.

Monitoring of Patient Outcome

The endpoint of a clinical trial is a measurable outcome that indicates the effectiveness of a treatment under evaluation. The endpoint is established prior to the commencement of the trial and will vary depending on the type and phase of the clinical trial. Examples of endpoints include, for example, tumour response rate – the proportion of trial participants whose tumour

was reduced in size by a specific amount, usually described as a percentage; disease-free survival – the amount of time a participant survives without cancer occurring or recurring, usually measured in months; overall survival – the amount of time a participant lives, typically measured from the beginning of the clinical trial until the time of death. For advanced and/or metastatic cancers, disease stabilisation – the proportion of trial participants whose disease has stabilised, for example, whose tumour(s) has ceased to grow and/or metastasise, can be used as an endpoint. Other endpoints include toxicity and quality of life.

Tumour response rate is a typical endpoint in Phase II trials. However, even if a treatment reduces the size of a participant's tumour and lengthens the period of disease-free survival, it may not lengthen overall survival. In such a case, side effects and failure to extend overall survival might outweigh the benefit of longer disease-free survival. Alternatively, the participant's improved quality of life during the tumour-free interval might outweigh other factors. Thus, because tumour response rates are often temporary and may not translate into long-term survival benefits for the participant, response rate is a reasonable measure of a treatment's effectiveness in a Phase II trial, whereas participant survival and quality of life are typically used as endpoints in a Phase III trial.

PHARMACEUTICAL KITS

The present invention additionally provides for therapeutic kits containing the antisense oligonucleotide and one or more chemotherapeutic agents in pharmaceutical compositions for use in the treatment of cancer. Individual components of the kit would be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

When the components of the kit are provided in one or more liquid solutions, the liquid solution can be an aqueous solution, for example a sterile aqueous solution. In this case the container means may itself be an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the composition may be administered to a patient.

The components of the kit may also be provided in dried or lyophilised form and the kit can additionally contain a suitable solvent for reconstitution of the lyophilised components. Irrespective of the number or type of containers, the kits of the invention also may comprise an instrument for assisting with the administration of the composition to a patient. Such an instrument may be an inhalant, syringe, pipette, forceps, measured spoon, eye dropper or any such medically approved delivery vehicle.

The disclosure of all patents, publications, including published patent applications, and database entries referenced in this specification are specifically incorporated by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were specifically and individually indicated to be incorporated by reference.

To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

EXAMPLES

EXAMPLE 1: *In vivo* Testing of the Combinations of the Present Invention in Mouse Xenograft Models

A. HT-29 human colon cancer cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. After the size of tumor reached an approximate volume of 50 mm^3, 4 days post tumor cell injection, mitomycin C was administered by bolus infusion into the tail vein at days 4, 11 and 18 with a dose of 3.5 mg/kg/week. Antitumor effect of mitomycin C was further compared to that of GTI-2040 in combination with mitomycin C. GTI-2040 was administered by bolus infusion into the tail vein every day at 6 mg/kg and mitomycin C was administered intravenously at days 4, 11 and 18 with a dose of 3.5 mg/kg/week, one hour after the treatments with GTI-2040. Control animals received saline alone for the same period as GTI-2040. All treatments were stopped at day 22. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in

tumor weights with each bar representing mean tumor weight calculated from 5 animals. As illustrated, mitomycin C treatments resulted in significant delay of tumor growth compared to saline control. The antitumor effects elicited by the combination of GTI-2040 and mitomycin C were significantly more potent than those obtained using mitomycin C alone (see Figure 1).

B. HT-29 human colon cancer cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 5-6 week old female CD-1 nude mice. After the size of tumor reached an approximate volume of 100 mm^3, 7 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of CPT-11 alone or that of GTI-2040 in combination with CPT-11. CPT-11 was administered intraperitoneally for 5 days in a row from day 7-12 with a dose of 20mg/kg in 100 □l saline. All treatments were stopped at day 32. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 9 animals. As illustrated, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control. The delay in tumor growth achieved with GTI-2040 was superior to the inhibitory effects observed with CPT-11 alone. The combination treatments of GTI-2040 and CPT-11 showed excellent cooperative effects that are significantly more potent than either agent alone (see Figure 2).

C. Caki-1 human renal cancer cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female SCID mice. After the size of tumor reached an approximate volume of 200 mm^3, 7 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of two chemotherapeutic agents including 5-FU and vinblastin. 5-FU was administered intraperitoneally at days 7-13, 21-27 and 35-36 with a dose of 13 mg/kg/day, while vinblastin was administered intraperitoneally at days 7, 14, 21, 28 and 35 at a dose of 0.6mg/kg/week. Antitumor effects of each of these compounds were further compared to those of GTI-2040 in combination with 5-FU or with vinblastin. The two chemotherapeutic agents were applied as described above, one hour after the treatments with GTI-2040 when

combination treatments occurred on the same day. All treatments were stopped at day 36. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 5 animals. As illustrated, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control. The delay in tumor growth achieved with GTI-2040 was superior to the inhibitory effects observed with each of two chemotherapeutic compounds. The combination of GTI-2040 with 5-FU or vinblastin showed super-additive antitumor effects with complete responses (see Figure 3).

D. Figure 4 shows results from two independent experiments. In both experiments, PC-3 human prostatic cancer cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old male SCID mice. After the size of tumor reached an approximate volume of 50 mm^3, 14 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 18 times (left panel) or 17 times (right panel), respectively. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of mitoxantrone (novantrone®) alone or in combination. Mitoxantrone was administered intravenously once at the beginning of the treatments at a dose of 2 mg/kg (left panel) or once a week for four weeks at a reduced dose of 0.8 mg/kg (right panel). All treatments were stopped at day 50 (left panel) or 48 (right panel), respectively. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 5 (left panel) or 10 (right panel) animals. As illustrated in the left panel, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control. The delay in tumor growth achieved with GTI-2040 was similar to the inhibitory effects observed with mitoxantrone alone. The combination of GTI-2040 with mitoxantrone (GTI-2040 +) showed some additive antitumor effects. In the right panel, mitoxantrone alone resulted in significant delay of tumor growth and the combination therapy was significantly more potent than mitoxantrone monotherapy.

E. Figure 5 shows results from two independent experiments. In both experiments, DU145 human prostatic cancer cells (1X10[7] cells in 100 μl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old male SCID mice. After the size of tumor reached an approximate volume of 50 mm^3, 13 (left panel) or 11 (right panel) days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 15 times (left panel) or 14 times (right panel), respectively. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of mitoxantrone (novantrone®) alone or in combination. Mitoxantrone was administered intravenously once at the beginning of the treatments at a dose of 2 mg/kg (left panel) or once a week for four weeks at a reduced dose of 0.8 mg/kg (right panel). All treatments were stopped at day 42 (left panel) or 38 (right panel), respectively. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 5 (left panel) or 10 (right panel) animals. As illustrated in the left panel, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control. The delay in tumor growth achieved with GTI-2040 was similar to the inhibitory effects observed with mitoxantrone alone. The combination of GTI-2040 with mitoxantrone (GTI-2040 +) showed some additive antitumor effects. In the right panel, mitoxantrone alone resulted in significant delay of tumor growth and the combination therapy was significantly more potent than mitoxantrone monotherapy.

F. A2058 human melanoma cells (1X10[7] cells in 100 μl of PBS) were subcutaneously injected into the right flank of 6-7 week old female CD-1 nude mice. After the size of tumor reached an approximate volume of 100 mm^3, 6 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of dacarbazine (DTIC) alone or that of GTI-2040 in combination with DTIC. DTIC was administered intravenously for 5 days in a row from day 6-10 at a dose of 80mg/kg in 100 □l saline. All treatments were stopped at day 24. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 10 animals. As illustrated, GTI-2040

treatments resulted in significant delay of tumor growth compared to saline control. The delay in tumor growth achieved with GTI-2040 was superior to the inhibitory effects observed with DTIC alone. The combination treatments of GTI-2040 and DTIC showed excellent cooperative effects that are significantly more potent than either agent alone (Figure 6).

G. Figure 7 shows results from tthree independent experiments. MDA-MB-231 human breast cancer cells ($1X10^7$ cells in 100 μl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. After the size of tumor reached an approximate volume of 100 mm^3, 5 days post tumor cell injection, GTI-2040, or the scrambled control oligonucleotide (Scr) were administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of taxol or doxorubicin alone or in combination. Taxol was administered intravenously once a week at a dose of 10 mg/kg for three (top left) or four weeks (bottom panel). Doxorubicin was administered intravenously once a week at a dose of 5 mg/kg for first three weeks (top left panel) or for two weeks (bottom panel). All treatments were stopped at day 33 (top left panel) or at day 26 (bottom panel), respectively. A day after the last treatment, tumors were excised from the animals and their weights were measured. A standard bar graph was used to demonstrate the differences in tumor weights with each bar representing mean tumor weight calculated from 10 animals (top panels). In the bottom panel, antitumor activities were estimated by the inhibition of tumor volume, which was measured with calipers. Each point represents mean tumor volume calculated from 10 animals per experimental group. As illustrated, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control in all three experiments. The delay in tumor growth achieved with GTI-2040 was superior to the inhibitory effects observed with taxol or doxorubicin alone. The combination therapy of GTI-2040 with taxol or doxorubicin was significantly more potent than either monotherapy. The top right panel demonstrates that a control oligonucleotide that has the same base composition as GTI-2040, but is not complementary to R2 mRNA has no significant anti-tumor activity as a monotherapy and it does not cooperate with doxorubicin, suggesting that the effects of GTI-2040 are sequence specific.

H. SK-OV-3 human ovary adenocarcinoma cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. After the size of tumor reached an approximate volume of 100 mm^3, 6 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 17 times. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of taxol or cisplatin alone or in combination. Taxol was administered intravenously once a week for first three weeks and intraperitoneally once a week for next two weeks at a dose of 10 mg/kg. Cisplatin was administered intravenously once a week for first three weeks and intraperitoneally once a week for next two weeks at a dose of 4 mg/kg. All treatments were stopped at day 40. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 9 animals per experimental group. As illustrated, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control. The delay in tumor growth achieved with GTI-2040 was similar or superior to the inhibitory effects observed with taxol or cisplatin alone, repectively. The combination therapy of GTI-2040 with taxol or cisplatin was significantly more potent than either monotherapy (Figure 8).

I. BxPC-3 human pancreatic carcinoma cells ($3X10^6$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female CD-1 nude mice. After the size of tumor reached an approximate volume of 100 mm^3, 21 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 17 times. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of Gemcitabine. Gemcitabine was administered intravenously every three days at a dose of 100 mg/kg. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 10 animals per experimental group. As illustrated, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control. As expected, treatment with Gemcitabine during the same period was ineffective against Gemcitabine-resistant tumor (Figure 9).

J. Hela S3 human cervix epitheloid carcinoma cells ($5X10^5$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female SCID mice. After the size of tumor reached an approximate volume of 100 mm^3, 3 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 6 times. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of Hydroxyurea or Cisplatin alone or in combination. Hydroxyurea was administered intraperitoneally every day at a dose of 250 mg/kg for 10 days. Cisplatin was administered intravenously once a week for three weeks at a dose of 4 mg/kg. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 10 animals per experimental group. As illustrated, GTI-2040 treatments resulted in significant delay of tumor growth compared to saline control. As expected, treatment with Hydroxyurea during the same period was ineffective against Hydroxyurea-resistant tumor. The delay in tumor growth achieved with GTI-2040 was superior to the inhibitory effects observed with Cisplatin alone, which was used as a positive control. The combination therapy of GTI-2040 with Hydroxyurea was only as effective as GTI-2040 monotherapy, as expected. The combination therapy of GTI-2040 with Cisplatin, however, was significantly more potent than either monotherapy (Figure 10).

K. MDA-CDDP-S4 human *in vivo*-selected Cisplatin-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 µl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female SCID mice. After the size of tumor reached an approximate volume of 100 mm^3, 7 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 9 times. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of Cisplatin or Taxol alone. Cisplatin was administered intravenously once a week for three weeks at a dose of 4 mg/kg. Taxol was administered intravenously once a week for three weeks at a dose of 10 mg/kg. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 10 animals per experimental group. As illustrated, GTI-2040 treatments caused significant reduction of tumor weight compared to saline control. As expected, treatment with Cisplatin during the same period was ineffective against Cisplatin-resistant tumor. The delay in tumor growth

achieved with GTI-2040 was similar to the inhibitory effects observed with Taxol, which was used as a positive control (Figure 11).

L. MDA-CDDP-S4 human *in vivo*-selected Cisplatin-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 μl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female CB-17 SCID mice. After the size of tumor reached an approximate volume of 100 mm^3, 9 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of Taxol alone and in combination. Taxol was administered i.p. once a week at a dose of 10 mg/kg. Antitumor activities were estimated by the inhibition of tumor volume (top panel), which was measured with calipers. Each point represents mean tumor volume calculated from 10 animals per experimental group. Animals were sacrificed and tumor weights taken at the end of the study (bottom right). GTI-2040 treatments caused significant reduction of tumor weight compared to saline control. The delay in tumor growth achieved with GTI-2040 was superior to the inhibitory effects observed with Taxol, which was used as a positive control. The effects of combined treatment were greater than either treatment alone. This study was repeated with similar results (bottom left) (see Figure 12).

M. MDA-MB435-To.1 human Taxol-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 μl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female SCID mice. After the size of tumor reached an approximate volume of 100 mm^3, 20 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg 15 times. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of Cisplatin or Taxol alone. Cisplatin was administered intravenously once a week for four weeks at a dose of 4 mg/kg. Taxol was administered intravenously once a week for four weeks at a dose of 20 mg/kg. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 9-10 animals per experimental group. As illustrated, GTI-2040 treatments caused significant reduction of tumor weight compared to saline control. As expected, treatment with Taxol during the same period was ineffective against Taxol-resistant tumor. The delay in tumor growth achieved

with GTI-2040 was superior to the inhibitory effects observed with Cisplatin, which was used as a positive control (see Figure 13).

N. MDA-MB435-To.1 human Taxol-resistant breast adenocarcinoma cells ($4X10^6$ cells in 100 µl of PBS) were injected into the fat pad (inside of right leg) of 6-7 weeks old female CB-17 SCID mice. After the size of tumor reached an approximate volume of 100 mm^3, 17 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was compared to that of Cisplatin alone and in combination. Cisplatin was administered intravenously once a week for four weeks at a dose of 4 mg/kg. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 10 animals per experimental group. At the end of the study the animals were sacrificed and tumors weighed. As illustrated, GTI-2040 treatment caused significant reduction of tumor weight compared to saline control. The delay in tumor growth achieved with GTI-2040 was superior to the inhibitory effects observed with Cisplatin, which was used as a positive control. The combination of the two compounds produced anti-tumor efficacy that was superior to either one alone (see Figure 14).

O. Human taxol-resistant promyelocytic leukemia cells (HL-60) ($7X10^6$ cells in 100 µl of PBS) were injected into the right flank of 6-7 weeks old female SCID mice. After the size of tumor reached an approximate volume of 100 mm^3, 10 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. The anti-tumor effect of GTI-2040 was further compared to that of taxol. Taxol was administered i.p. once a week at a dose of 10 mg/kg. Anti-tumor activity was estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 10 animals per experimental group. In addition animals were sacrificed and tumor weights taken at the end of the study. GTI-2040 treatments caused significant reduction of tumor weight compared to saline control. As expected, treatment with taxol had no effect on tumor growth or weight (see Figure 15).

P. LS513 cells ($1X10^7$ cells in 100 µl of PBS) were subcutaneously injected into the right flank of 6-7 weeks old female SCID mice. After the size of tumor reached an approximate volume of 100 mm^3, 8 days post tumor cell injection, GTI-2040 was administered by bolus infusion into the tail vein every other day at 10 mg/kg. Control animals received saline alone for the same period. Antitumor effect of GTI-2040 was further compared to that of CPT-11 alone or in combination. CPT-11 was administered i.p. for 5 days at a dose of 20 mg/kg/day. Antitumor activities were estimated by the inhibition of tumor volume, which was measured with caliper. Each point represents mean tumor volume calculated from 10 animals per experimental group. Tumor weights were measured after animals were sacrificed at the end of the treatment. These cells are not resistant to CPT-11 which was used as a positive control. As illustrated, GTI-2040 treatment resulted in significant delay of tumor growth compared to saline control. GTI-2040 is as effective as CPT-11 and in combination the efficacy is significantly greater than either treatment alone (see Figure 16).

EXAMPLE 2: Pharmacokinetics in the Monkey

GTI-2040 was administered to monkeys by continuous intravenous infusion for 21 days followed by a 21-day recovery period. Twenty-eight monkeys were administered one of the following dose levels: vehicle control, 2, 10, or 50 mg/kg/day (24.6, 123, and 615 mg/m^2/day). Toxicokinetic samples were collected prior to the initiation of infusion, approximately 8, 24, 48, and 96 hours following the start of infusion, and on Day 20 prior to the change in dose-syringes.

Plasma GTI-2040 concentrations in the 2 mg/kg dose group could not be detected (limit of detection was 1.25 µg/mL). Plasma concentrations appear to reach steady state by eight hours after dosing. The mean C_{max} values of GTI-2040 for the 10 mg/kg and 50 mg/kg dose groups were 2.94 and 23.6 µg/mL, respectively. The median times at which C_{max} occurred (T_{max}) were 480 and 252 hours for the 10 mg/kg and the 50 mg/kg dose groups, respectively. The concentrations at steady state (Css) were determined to be 1.88 and 14.3 µg/mL, for the 10 mg/kg and the 50 mg/kg dose groups, respectively. Plasma clearance was 250 mL/hr/kg for the 10 mg/kg group and 160 mL/hr/kg for the 50 mg/kg group.

EXAMPLE 3: Clinical Trials using the Combinations of the Present Invention

Examples of possible clinical trials that could be conducted t test the combinations of the present invention are provided in Table 4.

Examples of clinical trials that are currently underway using AS-II-626-20 (GTI-2040) are provided in Table 5. The following describes the Protocols involved for each trial:

1. PROTOCOL LO1-1409 (RENAL CELL CARCINOMA)

Study Description:

AS-II-626-60 and capecitabine combination therapy in patients with advanced or metastatic renal cell carcinoma (Phase I/II)

Population: Advanced or metastatic renal cell carcinoma having failed standard therapy

Study regimen: AS-II-626-60 (CIV infusion)
+ capecitabine
cycles: 14 days + 7 days rest

Phase I / II

Status: Ongoing in Phase II

Dosing: GTI-2040 was administered as a continuous intravenous infusion for 21 days at a starting dose of 148.0 mg/m^2/day in combination with capecitabine administered orally at a fixed dose of 1660 mg/m^2/day (divided into two daily doses for 21 days) followed by 7 days of rest.

To date, data have been collected on 21 patients evaluable for tumor assessment. One patient is still receiving treatment after eight months of therapy with GTI-2040 and capecitabine. The majority of patients had failed two or more prior therapies before entering the study, exhibited extensive metastases, and were representative of a population with very poor prognostic outcome in renal cell cancer. In the present clinical study, few treatment-related toxicities outside of those already known to occur with the test drugs were observed. More than half of the 21 evaluable patients in this study exhibited disease stabilization, ranging up to eight months. Tumor shrinkages of index tumors compared to baseline measurements were observed in some patients.

2. PROTOCOL L6093 (BREAST)

Study Description

A Phase I Study of AS-II-626-60 and Capecitabine in the treatment of Metastatic Breast Cancer

Population: Breast cancer, metastatic and failing 2 or more prior regimens

Study regimen; AS-II-626-60 + Capecitabine
14 days in 21 day cycle

Subjects: 40 (2 Stages: 20 ea)

Phase II

Status: Design under review

3. PROTOCOL L6104 (NSCLC)

Study Description

A Phase I/II Trial of AS-II-626-60 and Docetaxel in Metastatic or Advanced Non-Small Cell Lung Cancer

Population: Metastatic or unresectable locally advanced NSCLC

Study regimen AS-II-626-60 + Docetaxel

Subjects: 42 (12 Phase I; 30 Phase II)

Phase I / II

Status: Design under review

4. PROTOCOL L6090 (SOLID TUMORS)

Study Description

A Phase I Study of AS-II-626-60 and Gemcitabine in Patients with Solid Tumors

Population: Solid tumors meeting standard phase I criteria

Study regimen AS-II-626-60 + Gemcitabine

Subjects: 34

Phase I

Status: Design under review

5. PROTOCOL L6108 (AML)

Study Description

A Phase I Dose Escalation Trial of AS-II-626-60 in combination with cytarabine in Acute Myeloid Leukemia

Population: Acute myeloid leukemia (2 cohorts stratified by age +/-55)

Study regimen AS-II-626-60 + cytarabine

Subjects: 30

Phase I

Status: Design under review

6. PROTOCOL L6099 (COLON)

Study Description

A Phase I Trial of AS-II-626-60, Oxaliplatin and Capecitabine in Refractory Unresectable Colorectal Cancer

Population: Refractory unresectable colorectal cancer

Study regimen AS-II-626-60
+ oxaliplatin & capecitabine

Subjects: 15-20

Phase I

Status: Design under review

7. PROTOCOL L6102 (PROSTATE)

Study Description

A Phase II Study of AS-II-626-60 and Docetaxel in Patients with Hormone-Refractory Prostate Cancer

Population: Hormone refractory prostate cancer

Study regimen AS-II-626-60 + Docetaxel

Subjects: 40

Phase II

Status: on hold pending EOP-1 and medication availability.

TABLE 2: Summary of AS-II-626-20 Treatment in Combination with Standard Chemotherapy Drugs

Tumour	Mouse	Treatment	Tumor weight as % of saline control
Caki (renal)	CD-1	GTI-2040 5-FU Vinblastine GTI-2040 + 5-FU GTI-2040 + Vinblastine	3.3 52 26 0 0
HT-29 (colon)	SCID	Mitomycin C GTI-2040 + Mitomycin C	15 0.8
HT29 (colon)	CD-1	GTI-2040 CPT-11 GTI-2040 + CPT-11	19 36 1.4
MDA-MB-231 (breast)	CD-1	GTI-2040 Taxol Doxorubicin GTI-2040 + Taxol GTI-2040 + Doxorubicin	12.6 58 41 1 4.8
A2058 (melanoma)	CD-1	GTI-2040 DTIC GTI-2040+DTIC	20 68 8
PC-3 (prostatic)	SCID	Novantrone GTI-2040 + Novantrone	67 21
DU145 (prostatic)	SCID	GTI-2040 Novantrone GTI-2040 + Novantrone	n.a. 41 40 60 4.6 23
**SK-OV-3 (ovary)	CD-1	GTI-2040 Taxol Cisplatin GTI-2040 + Taxol GTI-2040 + Cisplatin	42 49 67 24 27

Results shown are mean tumour weights presented as a percentage of saline treated controls. ** is tumour volume data as percentage of saline control.

TABLE 3: Summary of AS-II-626-20 Treatment of Drug Resistant Tumours

Tumour *resistance*	Mouse	Treatment	Tumor weight as % of saline control
LS513 (colon) *multi-drug resistant (CPT-11 sensitive)*	SCID	CPT-11 GTI-2040 GTI-2040 + CPT-11	47 49 3
MDA-CDDP-S4 (breast) *Cisplatin*	SCID	GTI-2040 Taxol Cisplatin	32 32 78
MDA-CDDP-S4 (breast) *Cisplatin*	SCID	GTI-2040 Taxol GTI-2040 + Taxol	18 41 26 61 1 12
MDA-MB435-To.1 (breast) *Taxol*	SCID	GTI-2040 Taxol Cisplatin	42 109 69
MDA-MB435-To.1 (breast) *Taxol*	CB-17/ SCID	GTI-2040 Cisplatin GTI-2040 + cisplatin	37 56 .22
HL-60 (leukemia) *Taxol*	SCID	GTI-2040 Taxol	38 119
BxPC-3 (pancreatic) *Gemcitabine*	CD-1	GTI-2040 Gemcitabine	6.8 83
Hela S3 (cervix) *hydroxyurea*	SCID	GTI-2040 Hydroxyurea Cisplatin GTI-2040+ Hydroxyurea GTI-2040 + Cisplatin	24 92 63 36 13

Results shown are mean tumour weights presented as a percentage of saline treated controls.

TABLE 4: Examples f Clinical Trials that can be C nducted using Antisense Oligonucleotide AS-II-626-60 [SEQ ID NO:1] in Combination with Various Chemotherapeutic Agents

DISEASE	Performance Status Organ Function	Prior Therapy	Trial Design Schedule
S lid tumors	PS ≥ 2 Must have adequate hematologic, renal, and metabolic function	No limit stated ≥ 4 wks since prior RT ≥ 3 wks since prior therapy (6 wks for nitrosourea or mitomycin C)	AS-II-626-60 : CIV d1-21 (148-185 mg/m2/day) Capecitabine PO BID d1-21 (500-1500 mg) 28-day cycle
Solid tumors	PS 0-2 Creatinine < 2.0 mg/dL SGOT < 2 x normal Bilirubin < 1.5 mg/dL WBC > 4000 PLT > 100,000	No limit stated ≥ 4 wks since prior tx	AS-II-626-60 : CIV d1-21 (100-185 mg/m2/day) Capecitabine PO BID d1-21 (1100-2000 mg/m2/day) 28-day cycle
Breast	PS 60-100% Must have adequate hematologic, renal, and hepatic function	≥ 2 prior regimens No prior capecitabine or 5FU unless in adjuvant setting	AS-II-626-60 : CIV d1-21 (74-185 mg/m2/day) Capecitabine: PO BID d8-21 (600-1000 mg/m2) 28-day cycle
Colorectal	PS 0-2 bilirubin ≤ 1.5 x ULN	1 prior CT or adjuvant	AS-II-626-60 : CIV d1-21 (185 mg/m2/day) Capecitabine: PO BID d1-14

DISEASE	Perf rmance Status Organ Function	Prior Therapy	Trial Design Schedule
	SGOT/SGPT ≤ 3 x ULN		(start at 850 mg/m2) 28-day cycle
Colorectal	PS 60-100% Must have adequate hematologic, renal, and hepatic function	No prior oxaliplatin	**AS-II-626-60 : CIV d1-21 (74-185 mg/m2/day)** **Capecitabine: PO BID d2-15 (600-1000 mg/m2)** **Oxaliplatin: IV d2 (130 mg/m2/day)** 28-day cycle
Solid tumors	PS 0-2 Abnormal organ function permitted	≥ 1 prior regimens No prior docetaxel	**AS-II-626-60 : CIV d1-21 (50-185 mg/m2/day)** **Docetaxel: IV weekly x 3 (30-35 mg/m2)** 28-day cycle
Solid tumors	PS 0-1 Abnormal organ function not permitted	No restrictions	**AS-II-626-60 : CIV d1-21 (148-185 mg/m2/day)** **Docetaxel: IV d1, 8, 15 (30-35 mg/m2)** 28-day cycle
NSCLC & other solid tumors	PS 0-1 Abnormal organ function not permitted	≤ 2 prior CT	**AS-II-626-60 : CIV d1-22 (1-5 mg/kg/day)** **Docetaxel: IV d8, 15, 22 (35 mg/m2)**

DISEASE	Performance Status Organ Function	Pri r Therapy	Trial Design Schedule
			28-day cycle
NSCLC	PS 0-2 Must have adequate hematological, renal, & hepatic function	Prior platinum-based CT req No prior taxane CT > 1 systemic tx not permitted ≥ 28 days since prior surgery or RT	**AS-II-626-60** : CIV d1-14 (74-185 mg/m2/day) **Docetaxel:** IV d3 1st cycle, d1 2nd cycle (60-75 mg/m2/day) 21-day cycle
Prostate	PS 0-2 Abnormal organ function not permitted	No prior CT Must have failed front-line hormonal treatment	**AS-II-626-60** : CIV d1-21 (3-5 mg/kg/day) **Docetaxel:** IV d1, 8, 15 (30-36 mg/m2/day) 28-day cycle
Genitourinary	PS 0-2 creatinine 2X ULN, liver function <1.5 ULN	3 prior CT	**AS-II-626-60** : CIV d1-21 (100-185 mg/m2/day) **Docetaxel:** IV (40-80 mg/m2) q 3 wks
Solid tumors	PS 0-2 Abnormal organ function not permitted	≤ 2 prior tx	**Schedule A:** **AS-II-626-60** : CIV d1-21 (90-190, RP2D mg/m2/day) **Gemcitabine:** IV d1, 8, 15 (1000-1200 mg/m2) **Schedule B:** **AS-II-626-60** : CIV d1-21 (RP2D) **Gemcitabine:** CIV d1, 8, 15

DISEASE	Performance Status Organ Function	Pri r Therapy	Trial Design Schedule
			(750-1200 mg/m2) 28-day cycles
Solid tumors	PS 0-2 Abnormal organ function not permitted	No restrictions	**AS-II-626-60** : CIV d2-21 cycle 1 (100-185 mg/m2); CIV d1-21 for subsequent cycles **Gemcitabine:** IV d1, 8, 15. (400-1000 mg/m2) 28-day cycle
Colon cancer	PS ≥ 60% survival ≥ 3 mo Must have adequate hematologic, renal, and hepatic function	Must have progressed following 5FU, irinotecan, & oxaliplatin CT	**AS-II-626-60** : CIV d1-21 (85-185 mg/m2/day) **Gemcitabine:** IV d1, 8 q 6 wks (1000 mg/m2) **Capecitabine:** PO BID d1-14 q 3 wks (650 mg/m2)
NSCLC	PS > 60% survival > 3 mo Must have adequate hematologic, renal, and hepatic function	No prior gemcitabine or AS-II-626-60 ≥ 4 wks since prior CT ≥ 6 wks since prior mitomycin C CT ≥ 2 wks since prior RT	**AS-II-626-60** : CIV d1-21 (74-185 mg/m2/day) **Gemcitabine:** IV d2, 9, 16 (800-1000 mg/m2) 28-day cycle
Renal cell carcinoma	PS 0-2 Must have adequate	**Phase 1** No prior gemcitabine CT **Phase 2**	**AS-II-626-60** : CIV d1-21 (111-185 mg/m2/day) **Gemcitabine:** IV weekly x 3

DISEASE	Performance Status Organ Function	Prior Therapy	Trial Design Schedule
	hematologic, renal, and hepatic function	≤ 2 prior tx allowed ≥ 4 wks since prior RT, surgery, or tx	(800-1000 mg/m2) 28-day cycle
Breast cancer	PS 60-100% Must have adequate hematologic, renal, and hepatic function	≤ 1 prior tx no prior platinum or gemcitabine allowed	**AS-II-626-60** : CIV d1-21 (74-185 mg/m2/day) **Gemcitabine:** IV d2, 9 (600-1000 mg/m2/day) **Oxaliplatin:** IV d2 (130 mg/m2/day) 28-day cycle
AML	PS 0-2 Abnormal organ function permitted: Cr ≤ 2.0 mg/dL total bilirubin < 2.0 mg/dL AST/ALT < 5 x ULN	Prior therapy O.K.	**AS-II-626-60** : CIV d1-21 (120-280 mg/m2/day) **Idarubicin:** IV dx3 (12 mg/m2/day)
AML	PS 0-2 Abnormal organ function permitted: Cr ≤ 2.0 mg/dL total bilirubin ≤ 2.0 mg/dL	Prior therapy O.K.	**AS-II-626-60** : CIV d1-21 (120-280 mg/m2/day) **Ara-C:** IV dx4 (1 g/m2/day)
AML, CML	PS 0-2	At least 1 prior therapy	**AS-II-626-60** : CIV d1-5 (2-

DISEASE	Performance Status Organ Function	Prior Therapy	Trial Design Schedule
	Cr < 2, bilirubin < 2		10 mg/kg/day) **Ara-C:** IV d1-5 (1 g/m2/day)
AML	PS ECOG 0-2 Abnormal organ function not permitted	Chemotx, including auto or allo SCT	**AS-II-626-60 :** CIV d1-8 (3.5, 5 mg/kg/day) **Mitoxantrone:** IV d4-8 (6 mg/m2/day) **Etoposide:** IV d4-8 (80 mg/m2/day) **Ara-C:** IV d4-8 (500 -1000 mg/m2/day)
CML	PS 0-2 Abnormal organ function permitted: Cr ≤ 2.0 mg/dL total bilirubin < 2.0 mg/dL AST/ALT < 5 x ULN	Imatinib mesylate failed	**AS-II-626-60 :** CIV d1-21 (120-280 mg/m2/day) **Fludarabine:** IV d3-7 (30 mg/m2/day) **Ara-C:** IV d3-7 (2 g/m2/day) **Filgrastim:** 5 mcg/kg/day, start d8
Metastatic cancer	ECOG ≤ 2 Must have adequate hematologic, renal, and hepatic function	≥ 4 wks since prior RT **Phase 1:** unlimited chemotherapy **Phase 2:** must have prior paclitaxel and carboplatin	**AS-II-626-60 :** CIV d1-14 (125-185 mg/m2/day) **Carboplatin:** AUC = 5, 6 **Paclitaxel:** IV weekly (135-175 mg/m2) Cycles repeat q 21 days
Head & Neck, esophagus,	PS 0-2	One prior multimodality tx permitted (incl platinum-based)	**AS-II-626-60 :** CIV d1-21 (dose TBD)

DISEASE	Perf rmance Status Organ Function	Prior Therapy	Trial Design Schedule
lung	Abnormal organ function not permitted		**Cisplatin:** IV weekly (15-40 mg/m2) 28-day cycle
SCLC	PS 0-2 Abnormal organ function not permitted	No prior chemotx	**AS-II-626-60** : CIV d1-21 (3-5 mg/kg/day) **Cisplatin:** IV d1 (50-60 mg/m2/day) **Irinotecan:** IV d1, 8, 15 (50-60 mg/m2/day)
Pancreatic adenocarc	PS 0-2 Abnormal organ function not permitted	Prior GEM required; ≥ 4 wks since GEM regimen No prior irinotecan allowed	**AS-II-626-60** : CIV d1-14 (104-185 mg/m2/day) **Irinotecan:** IV d1, 8 (75-125 mg/m2/day) 21-day cycle
Pancreas, gall bladder, & biliary ducts	PS 0-2 Total bilirubin < 3X ULN eligible	1 prior tx: either GEM or bryo/Taxol	**AS-II-626-60** : CIV d1-21 (dose TBD) **5-FU:** CIV d1-21 (100-225 mg/m2/day) 28-day cycle
Pancreas cancer	PS = ≥ 60% AGC > 1,500/mcL Hgb ≥ 9 mg/dL plts > 100,000/mcL	Must have PD after previous GEM chemotx for metastatic pancreatic cancer	**AS-II-626-60** : CIV d1-21 (85-185 mg/m2/day) **Oxaliplatin:** IV d1 (130 mg/m2/day) **Capecitabine:** PO BID d1-14

DISEASE	Performance Status Organ Function	Prior Therapy	Trial Design Schedule
	creat ≤ 1.5 ULN bilirubin ≤ 1.5 ULN ALP/SGOT/SGPT ≤ 3 x iULN		(1000 mg/m2/day) Cycles repeat q 21 days Sched modified if signif tox in 2/3 pts @ dose level 1: **AS-II-626-60** : CIV <u>d1-14</u> (85-185 mg/m2/day) **Oxaliplatin** and **capecitabine** as above Cycles repeat q 21 days
Prostate cancer	PS ≤ 2 ANC ≥ 1.5 x 10e9/L plts ≥ 100 x 10e9/L creat ≤ 2 x ULN or CrCl ≥40 mL/min bili ≤1.5 x ULN AST/ALT ≤3xULN LVEF ≥50%	Hormone-refractory; no prior chemotx; ≥4 wks since prior XRT	**AS-II-626-60** : 111-185 mg/m2/day CIV d1-14 **Mitoxantrone**: 12 mg/m2 IV q3w **Prednisone**: 5 mg PO BID Cycles repeat q 21 d to 10 cycles or PD

TABLE 5: Current Clinical Trials using Antisense Oligonucleotide AS-II-626-60 [SEQ ID NO:1] in Combination with Various Chemotherapeutic Agents

COMPOUND/ PROTOCOL	PHASE/ NO. OF PTS	TARGET TUMOR	TREATMENT REGIMEN
AS-II-626-60 Protocol LO1-1409	I / N=9 II / N=43	Renal cell (advanced/ metastatic, prior Rx)	AS-II-626-60 + Capecitabine
AS-II-626-60 Protocol L6093	II / N=40 (N=20 per stage)	Breast (metastatic; 2 prior Rx)	AS-II-626-60 + Capecitabine
AS-II-626-60 Protocol L6104	I / N=12 II / N=30	NSCLC (metastatic / unresectable & advanced)	AS-II-626-60 + Docetaxel
AS-II-626-60 Protocol L6090	I / N=34	Solid tumors	AS-II-626-60 + Gemcitabine
AS-II-626-60 Protocol L6108	I / N=30	Acute myeloid leukemia (2 cohorts by age +/- 55)	AS-II-626-60 + Cytarabine (+ Mitoxantrone & Etoposide tbd)
AS-II-626-60 Protocol L6099	I / N=19	Colorectal (refractory, unresectable)	AS-II-626-60 + Oxaliplatin & Capecitabine
AS-II-626-60 Protocol L6102	II / N=40	Prostate	AS-II-626-60 + Docetaxel

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A use of an antisense oligonucleotide comprising SEQ ID NO:1 in combination with capecitabine for the treatment of renal carcinoma.

ABSTRACT

The present invention provides combinations of antisense oligonucleotides directed to a mammalian ribonucleotide reductase R2 gene and one or more chemotherapeutic agents for use in the treatment of cancer. The combinations of the present invention are more effective in decreasing the growth and/or metastasis of cancer cells, including drug resistant cancer cells, than treatment with the antisense oligonucleotide or the chemotherapeutic agent(s) alone.

Weight of Human Colon Adenocarcinoma (HT-29) in CD-1 Nude Mice Treated with Combination Therapy

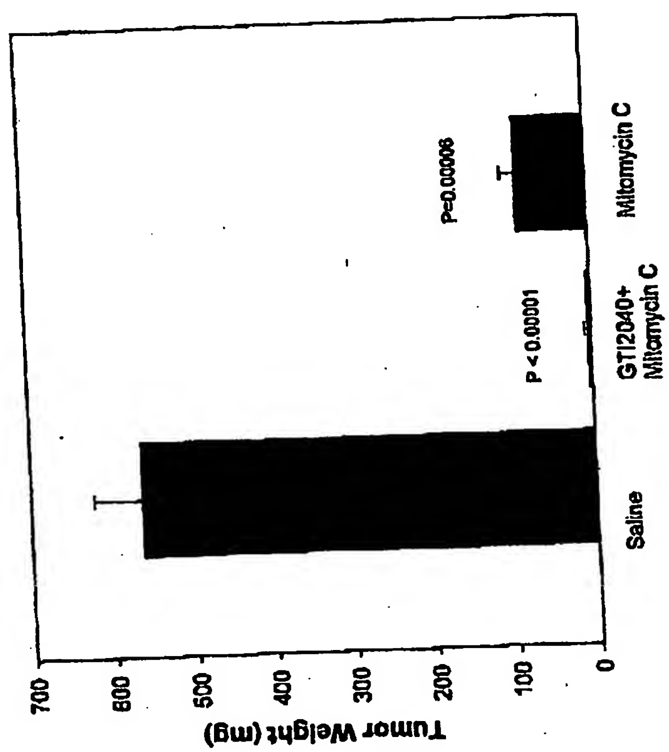


Figure 1

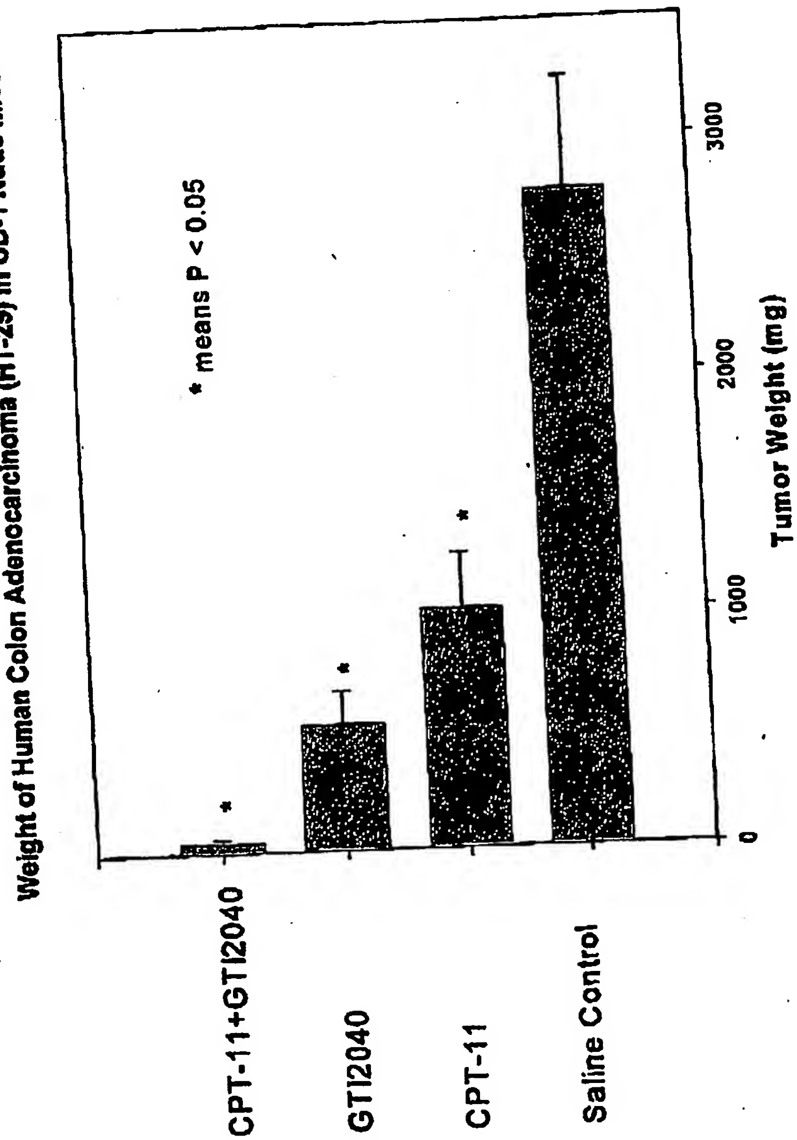
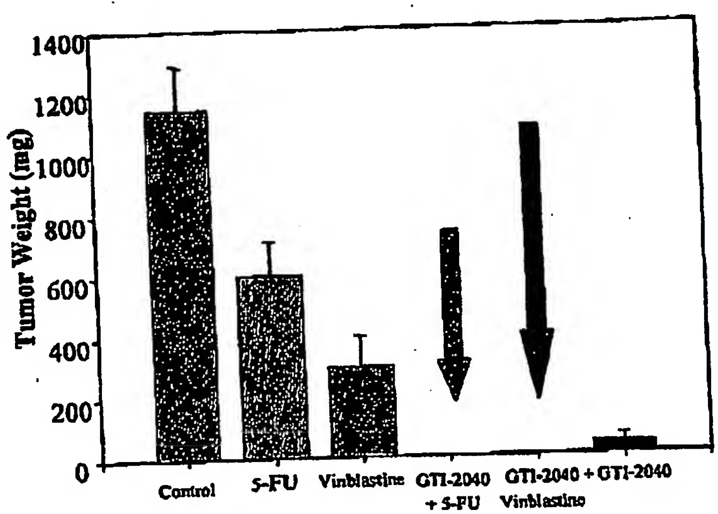

Weight of Human Colon Adenocarcinoma (HT-29) in CD-1 Nude Mice
* means P < 0.05
CPT-11+GTI2040
GTI2040
CPT-11
Saline Control
0
1000
2000
3000
Tumor Weight (mg)

Figure 2

Pre-clinical Efficacy in Combination Therapy: Kidney Tumors (Caki-1)

A



B

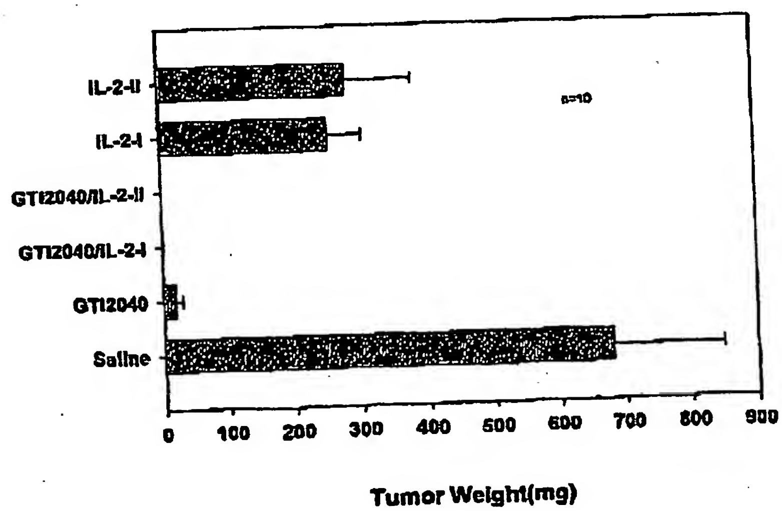


Figure 3

Weight of Human Prostate Carcinoma (PC-3) in SCID Mice

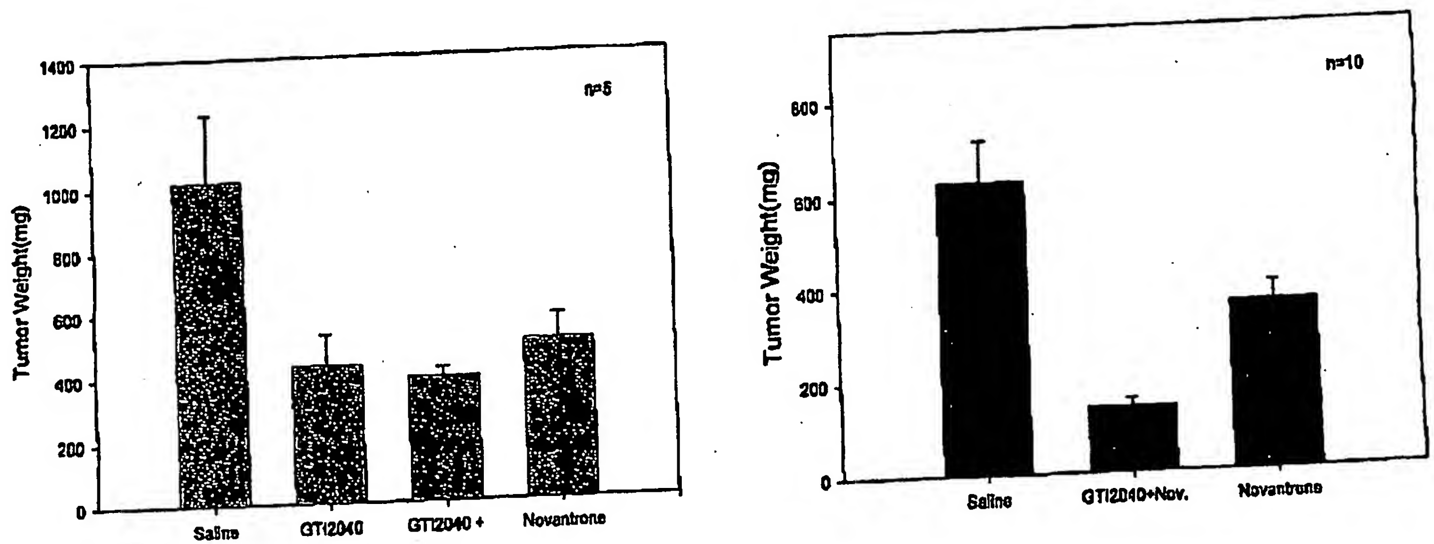


Figure 4

Weight of Human Prostate Carcinoma(DU145) in SCID Mice

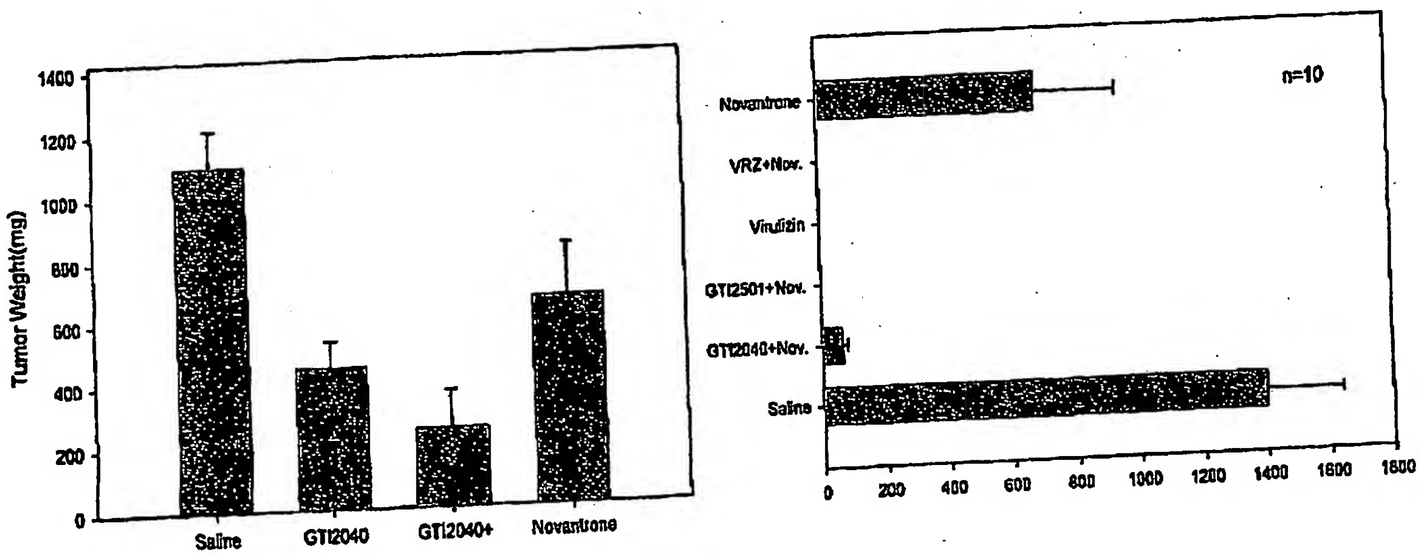


Figure 5

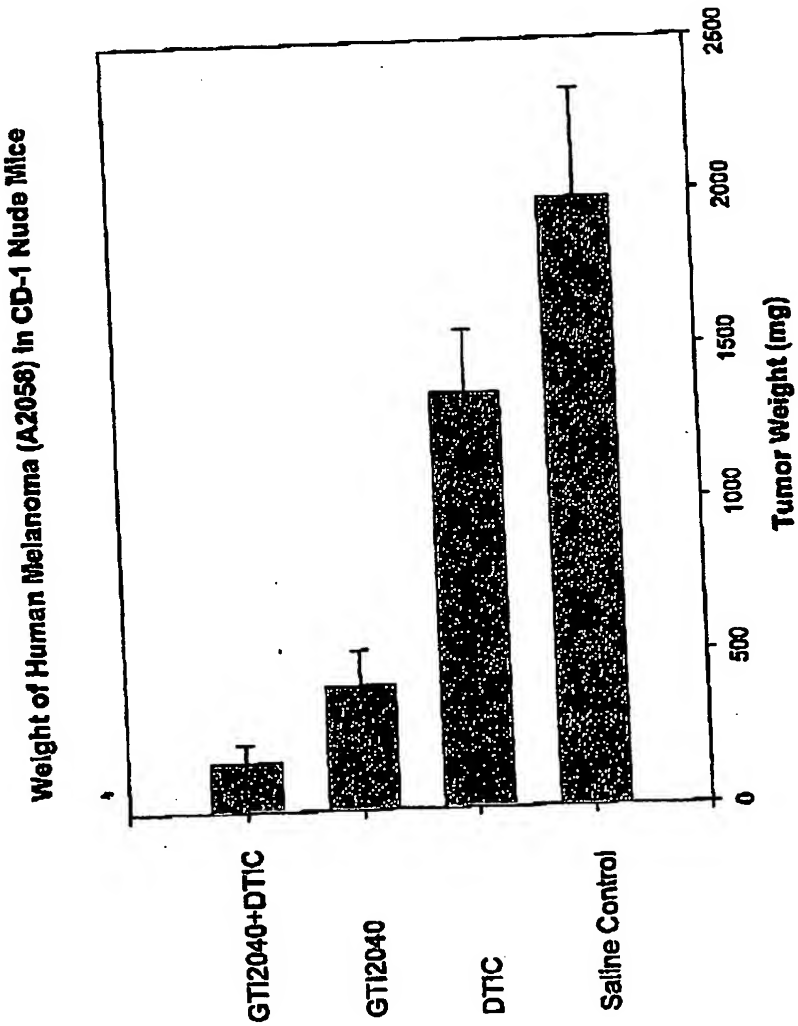
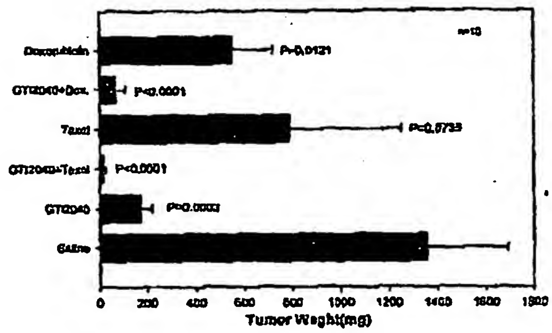
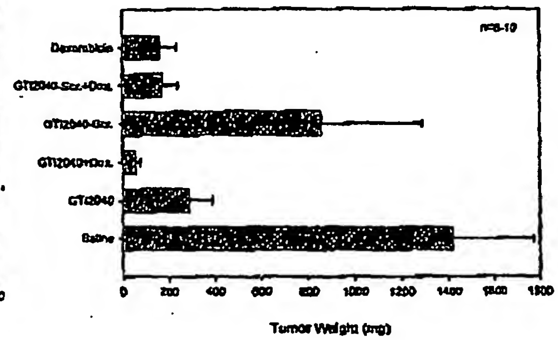

Weight of Human Melanoma (A2058) in CD-1 Nude Mice
GTI2040+DTIC
GTI2040
DTIC
Saline Control
0
500
1000
1500
2000
2500
Tumor Weight (mg)

Figure 6

Weight of Human Breast Adenocarcinoma (MDA-MB-231) in CD-1 Nude Mice





Growth of Human Breast Adenocarcinoma (MDA-MB-231) in CD-1 Nude Mice

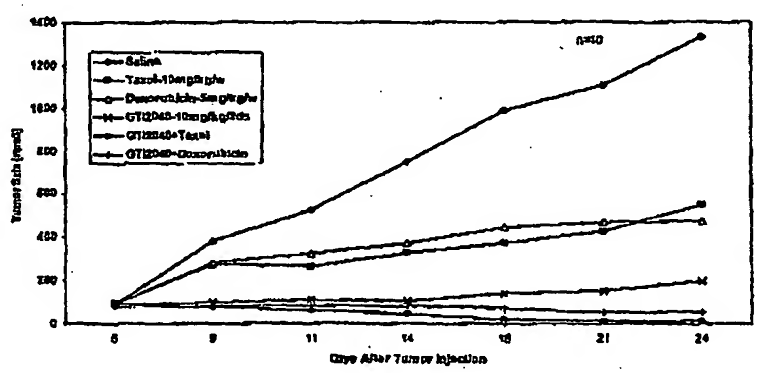


Figure 7

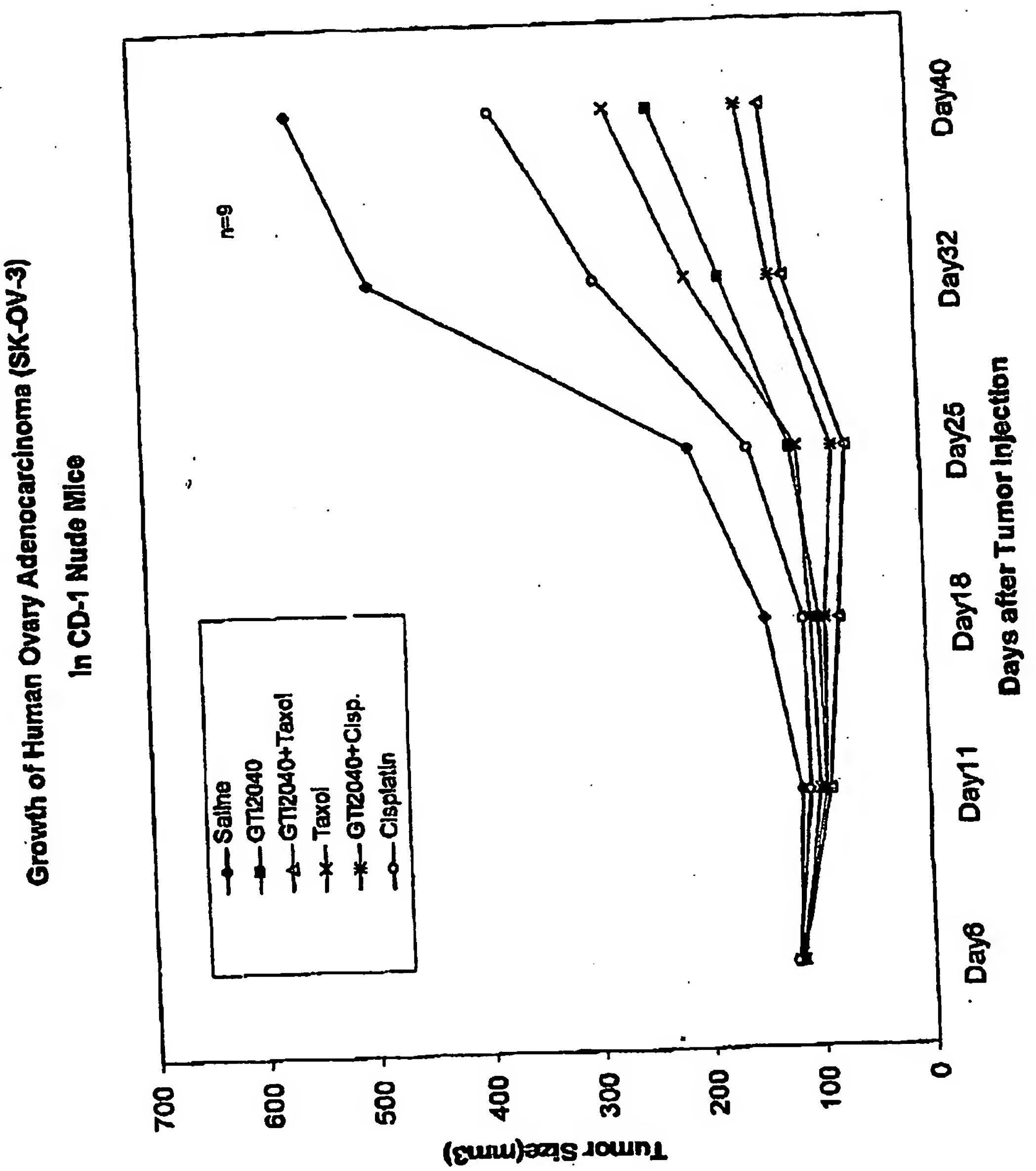
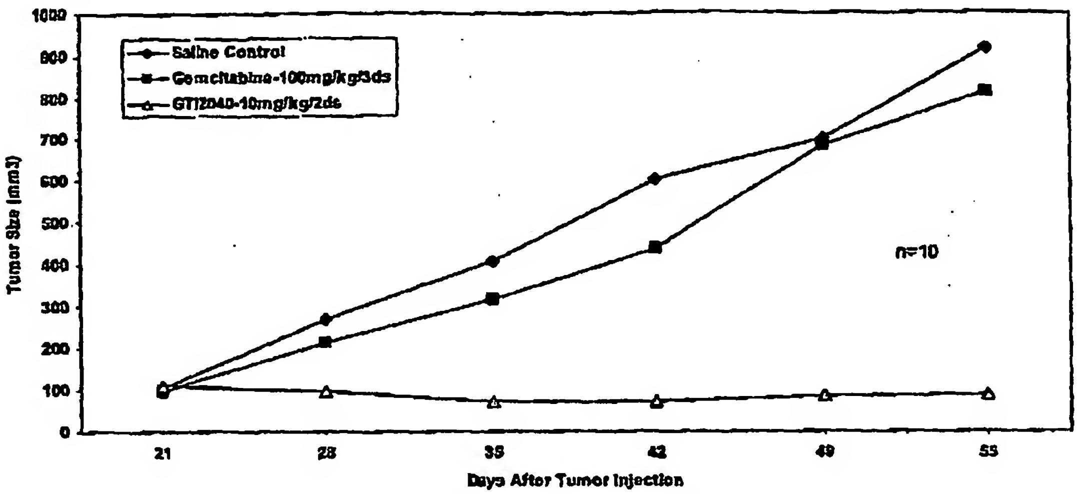
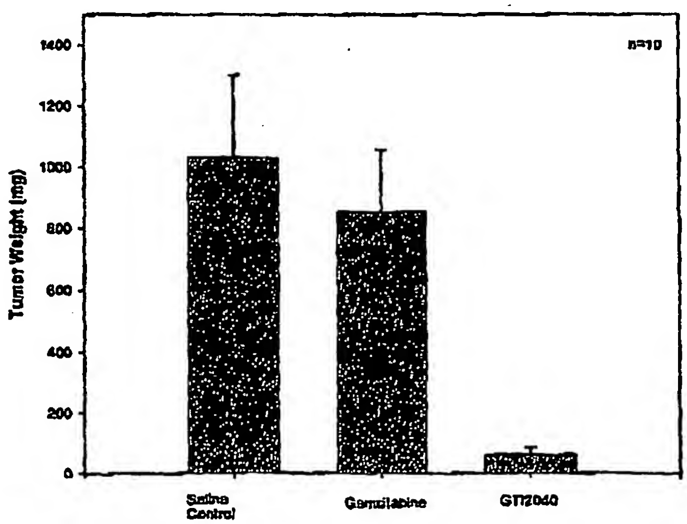

Growth of Human Ovary Adenocarcinoma (SK-OV-3)
In CD-1 Nude Mice
Saline
GTI2040
GTI2040+Taxol
Taxol
GTI2040+Cisp.
Cisplatin
n=9
Tumor Size(mm3)
700
600
500
400
300
200
100
0
Day6
Day11
Day18
Day25
Day32
Day40
Days after Tumor Injection

Figure 8

Growth of Human Pancreatic Carcinoma (BxPC-3) in CD-1 Nude Mice



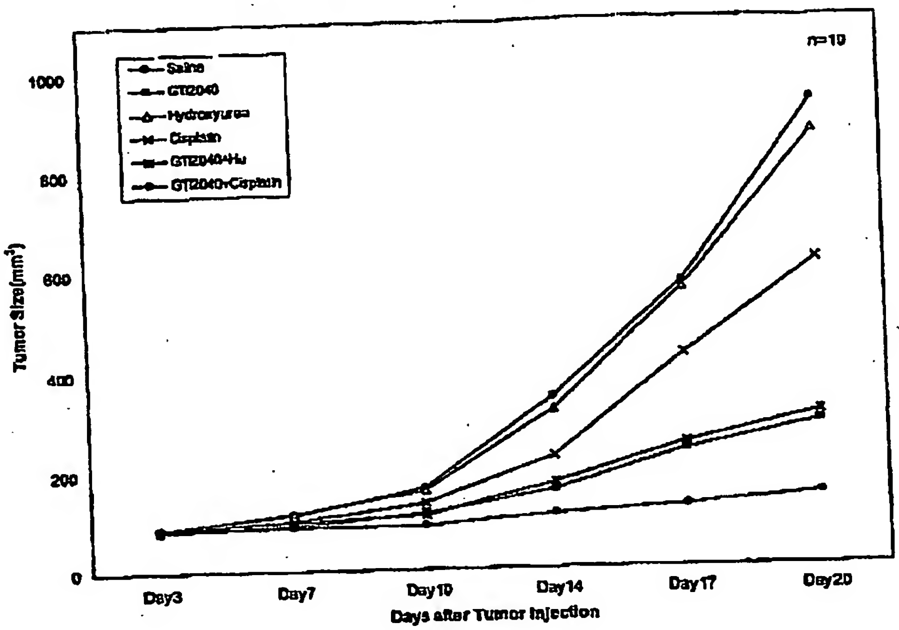
Weight of Human Pancreatic Carcinoma (BxPC-3) in CD-1 Nude Mice



Statistical Analysis:	P value
Saline : Gemcitabine	0.3963
Saline : GTI2040	<0.0001

Figur 9

Growth f Human Cervix Epitheloid Carcinoma
(Hela S3-HU-Resistanc) in SCID Mic



Weight of Human Cervix Epitheloid Carcinoma
(Hela S3-HU-Resistance) in SCID Mice

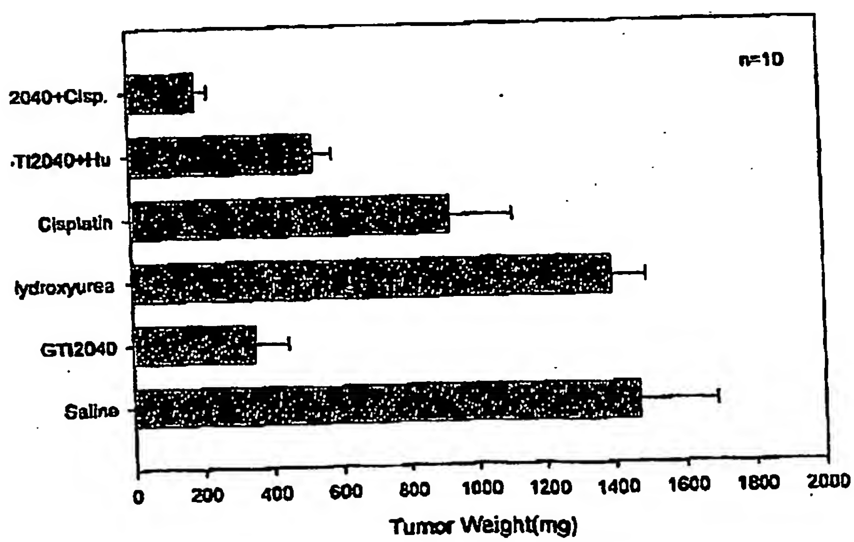
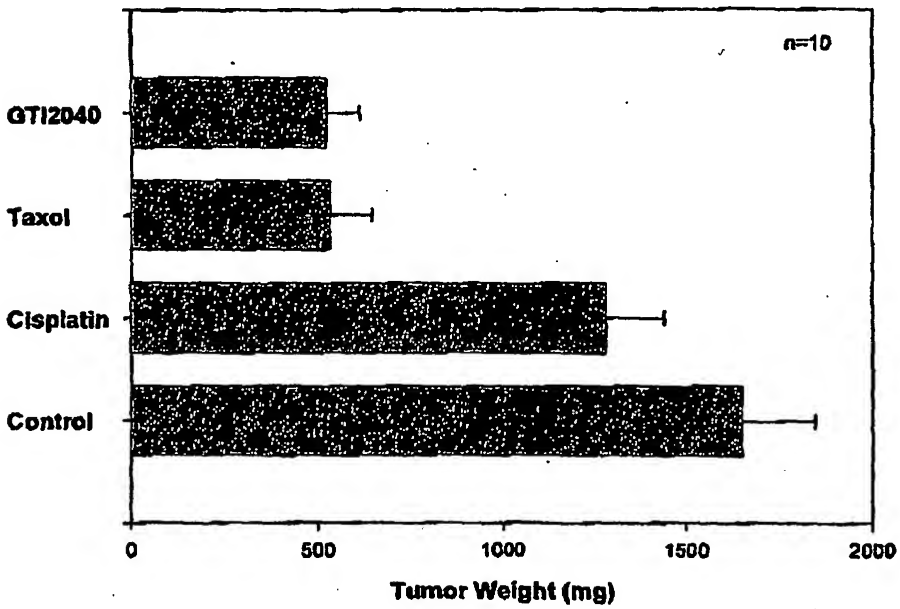


Figure 10

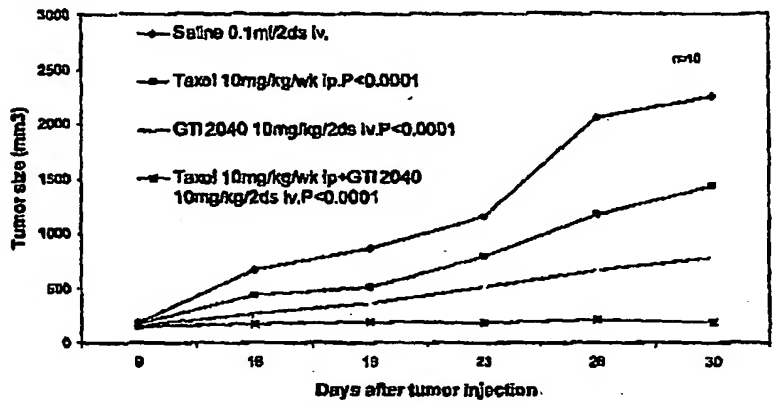
Weight of Human Cisplatin-Resistant Breast Adenocarcinoma Implanted at the Fat Pad of SCID Mice



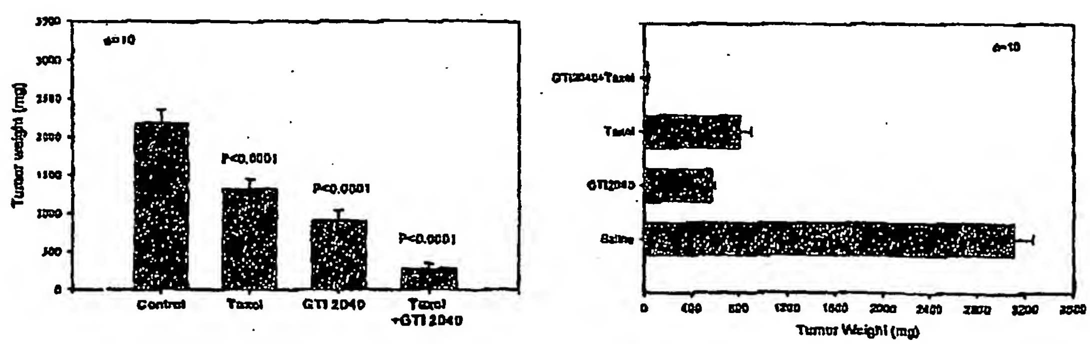
Statistical Analysis:	P value
Saline : Cisplatin	0.0834
Saline : Taxol	<0.0001
Saline : GTI2040	<0.0001
GTI2040 : Cisplatin	0.0007
GTI2040 : Taxol	0.9547

Figure 11

Growth Of Human Breast Cancer (MDA-CDDP-S4) In CB-17 SCID Mice Treated With Taxol, GTI 2040, and Taxol+GTI-2040 (Orth topical transplant)



Tumor Weight Of Human Breast Cancer (MDA-CDDP-S4) In CB-17 SCID Mice Treated With Taxol, GTI 2040, and Taxol+GTI-2040



GTI 2040 : Taxol P=0.0135
GTI 2040 : GTI 2040+Taxol P=0.0003
Taxol : GTI 2040+Taxol P<0.0001

Figure 12

W ight of Human Tax l-Resistant Breast Ad nocarcinoma (MDA-MB435-To.1) Implanted at th Fat Pad f SCID Mice

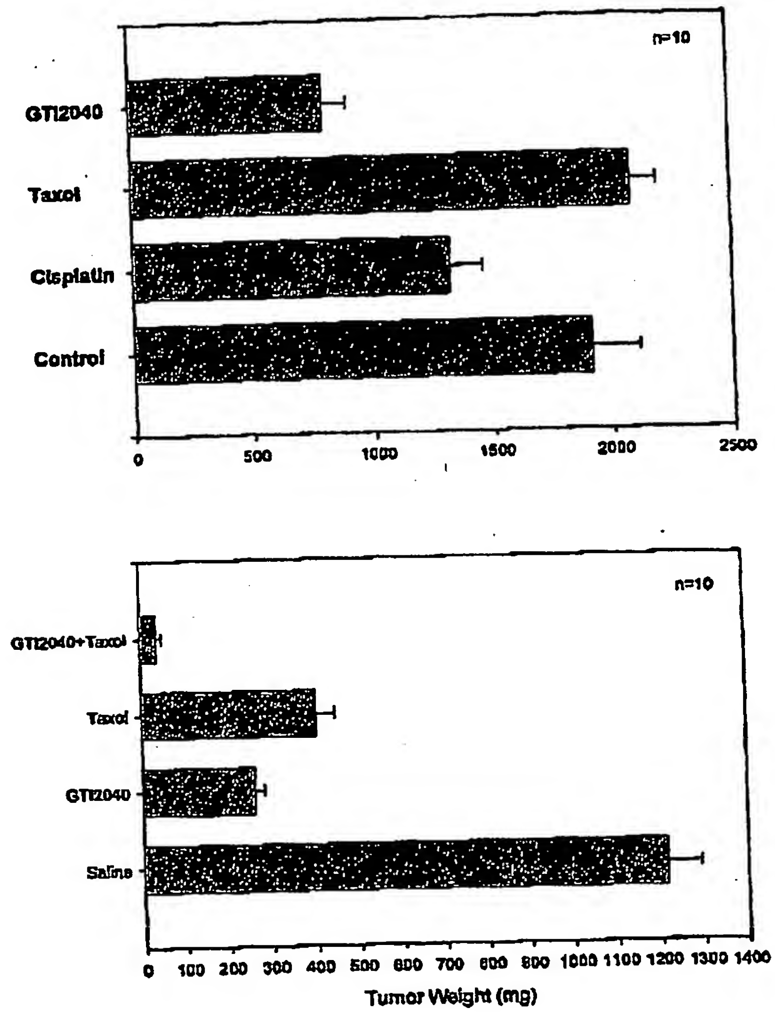
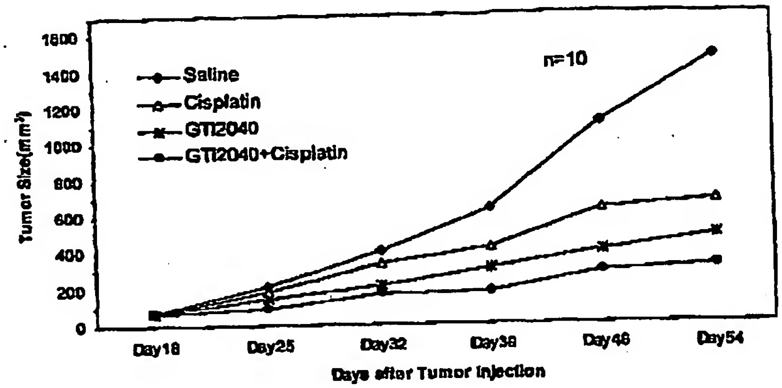


Figure 13

Growth f Human Breast Ad n carcinoma (MDA-MB435-To. 1)
in SCID Mice



Weight of Human Breast Adenocarcinoma (MDA-MB-435-To. 1)
in SCID Mice

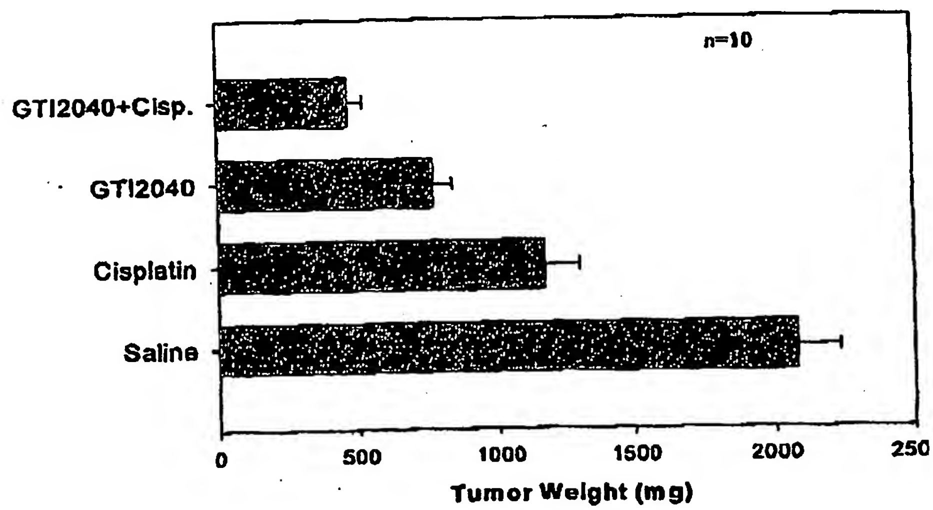
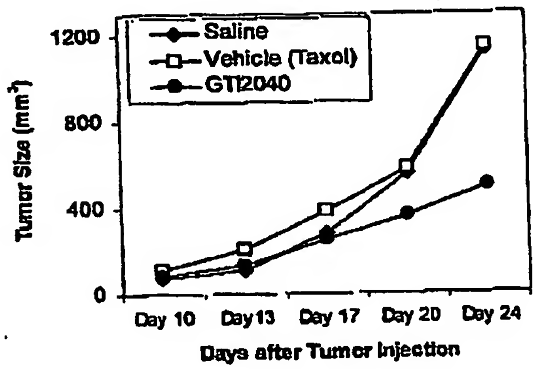


Figure 14

Growth f Pr myel cytic Leuk mia HL-60
(Tax l-Resistant) in SCID Mic



Weight of Human Promyelocytic Leukemia HL-60
(Taxol-Resistant) in SCID Mice

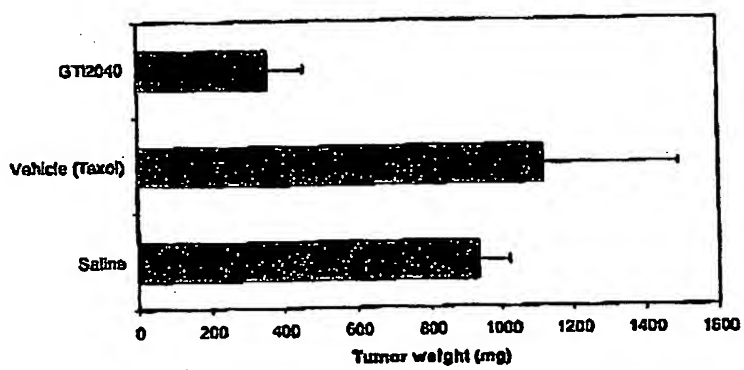
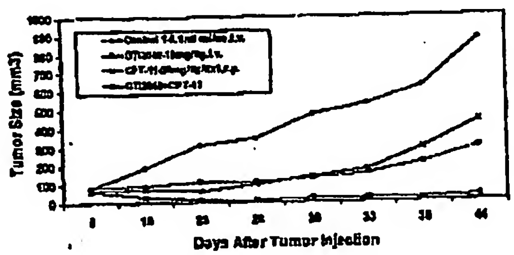
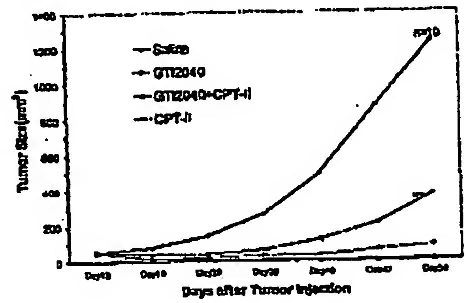


Figure 15

Growth of Human Multi-Drug Resistance Colon Adenocarcinoma (LS513) in SCID Mice





Weight of Human Colon Multi-Drug Resistance Carcinoma (LS513) in SCID Mice

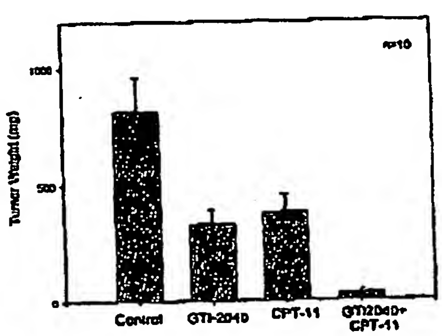


Figure 16